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ERRATA INDEX

Erratum. In the article "A Comparison of Marijuana Users and Non-users" by Norman E. Zinberg and Andrew T. Weil (*Nature*, **226**, 119; 1970), the third sentence in the first column on page 122 should be "There were no signs of overt intellectual deterioration in the C interviewees, . . .".

Erratum. Some corrections should be made to the article "Can Water Polymerize? An Inquiry into the Possible Existence of Strong Bonds between Water Molecules" by Allen P. Minton (*Nature*, **226**, 151; 1970). The first sentence of the second paragraph (p. 151) should read "A modified version of the Iterative Extended Huckel Theory (IEHT)[7,8] has been used to make electronic structure calculations . . .". Equation (1) should read:

$$\mathcal{E}_c = n\mathcal{E}_i + \sum_{i=1}^{n} \mathcal{D}_i + \sum_{i=1}^{n}\sum_{j=i+1}^{n} \mathcal{H}_{ij}$$

On page 152, paragraph 3, line 2, the reference to previous calculations on the interaction between water molecules, given as ref. 9, should be replaced by the additional reference 11: Hankins, D., Moskowitz, J. W., and Stillinger, F. H., *Chem. Phys. Lett.*, **4**, 527 (1970).

Erratum. The title of the communication by Mepham and Lane (*Nature*, **226**, 180; 1970) was given incorrectly on the contents page. It should read: "Adaptive significance of the sculptured exine of pollen".

Erratum. In Fig. 2 of the article "Preliminary Geological Interpretation of a New Hominid Site in the Lake Rudolf Basin" by A. K. Behrensmeyer (*Nature*, **226**, 225; 1970), the K–Ar date for the tuff in Koobi Fora should be 2·6 ± 0·3 m.y.

Erratum. In the article "Characterization of Ovine Hypothalamic Hypophysiotropic TSH-releasing Factor" by Roger Burgus, Thomas F. Dunn, Dominic Desiderio, Darrell N. Ward, Wylie Vale and Roger Guillemin (*Nature*, **226**, 321; 1970), corrections should be made to Tables 5 and 6. The first column of Tables 5 and 6 should read respectively:

Saline	Saline
Saline	5×10^{-9} g (X)
Saline	20×10^{-9} g (X)
Saline	80×10^{-9} g (X)
150×10^{-9} g (X)	T_3, saline
150×10^{-9} g (X)	T_3, 5×10^{-9} g (X)
150×10^{-9} g (X)	T_3, 20×10^{-9} g (X)
150×10^{-9} g (X)	T_3, 80×10^{-9} g (X)
	T_3, 1×10^{-6} g (X)

Erratum. The following corrections should be made in the article "Increase in Antiviral Activity of Polynucleotides by Thermal Activation" by E. de Clercq, R. D. Wells and T. C. Merigan (*Nature*, **226**, 364; 1970): The second sentence of the first paragraph should read "The thermal activating effect . . . was most marked with the alternating polyribonucleotides such as poly (A–U) and poly (I–C)". The second footnote in Table 1 should read "†Minimal Eagle's medium (MEM), *p*H 7·0–7·5 . . . ". In the paragraph following Table 1, the first sentence should read "Preincubation at 37° C, compared with 0° C, rendered all polynucleotides, including poly I·poly C, significantly more active in reducing virus plaque formation in human cells, when the incubation was carried out in MEM (Table 1)", and the sentence beginning on line 16 of that paragraph should read "Addition of 10^{-3} M EDTA following the 37° C incubation step or rapid cooling to 0° C (by immersion in solid CO_2–ethanol) did not reverse the activating effect . . .".

Erratum. The contents entry for the article by N. R. Blacklow and A. Z. Kapikian "Serological Studies with EB Virus in Infectious Lymphocytosis" (*Nature*, **226**, 647; 1970) should have been **Infectious lymphocytosis,** not **Infectious mononucleosis.** In the article itself, five lines from the end, read "these children" not "three children".

Erratum. An amendment should be made to the erratum (*Nature*, **226**, 782; 1970) referring to the article "Increase in Antiviral Activity of Polynucleotides by Thermal Activation" by E. de Clercq, R. D. Wells and T. C. Merigan (*Nature*, **226**, 364; 1970). The sentence beginning on line 16 of the paragraph following Table 1 should read "Addition of 10^{-3} M EDTA following the 37° C incubation step did reverse the activating effect whereas rapid cooling to 0° C did not".

Corrigendum. In the article "Netherlands: Stop and Go on the Nuclear Merry-go-round" (*Nature*, **226**, 1020; 1970), the figure given in the right-hand column of p. 1021, line 22, should be Dfl. 550,000,000, not Dfl. 550,000.

Erratum. The paper referred to as by E. P. Geiduschek *et al.* in the report of the RNA transcription symposium at Cold Spring Harbor (*Nature*, **226**, 1094; 1970) should be attributed to A. Cascino, S. Riva and E. P. Geiduschek.

Erratum. In the article "Solar UV Flash Spectrum 1400 Å–1960 Å" by G. E. Brueckner, J. F. Bartoe, K. R. Nicolas and R. Tousey (*Nature*, **226**, 1132; 1970), the following corrections should be made. In line 4 of paragraph 1, XUV should read "extreme ultraviolet"; the reference to Fig. 6 on line 57, p. 1134, should be to Fig. 5; column 3 of Table 2 should read as follows:

	Transition	
2p	$^3P_2$–2p′	$^3D_3$
	$^3P_2$–	$^3D_1$
	$^3P_2$–	$^3D_2$
	$^3P_1$–	$^3D_1$
	$^3P_1$–	$^3D_2$
	$^3P_0$–	$^3D_1$
2p	$^3P_2$–3s	$^1P_1$
	$^3P_1$–	$^1P_1$
	$^3P_0$–	$^1P_1$
2p	$^3P_2$–3s	$^3P_1$
	$^3P_1$–	$^3P_0$
	$^3P_1$–	$^3P_1$
	$^3P_2$–	$^3P_2$
	$^3P_0$–	$^3P_1$
	$^3P_1$–	$^3P_2$

Erratum. In the article "Advantages of an Antagonist" (*Nature*, **226**, 1199; 1970) two references were incorrectly cited. The correct citation for Obata *et al.* is *Exp. Brain Res.*, **4**, 43 (1967) and that attributed to Werman *et al.* (*Life Sci.*, **4** 2075; 1965) was actually due to Aprison and Werman.

Erratum. In the article "RNA-dependent DNA Polymerase in Virions of Rous Sarcoma Virus" by Howard M. Temin and Satoshi Mizutani (*Nature*, **226**, 1211; 1970), the third line of paragraph 4 should read "Tris-HCl buffer at *p*H 8·0, containing 0·33 mM EDTA and".

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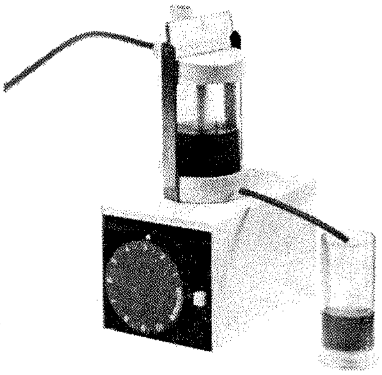

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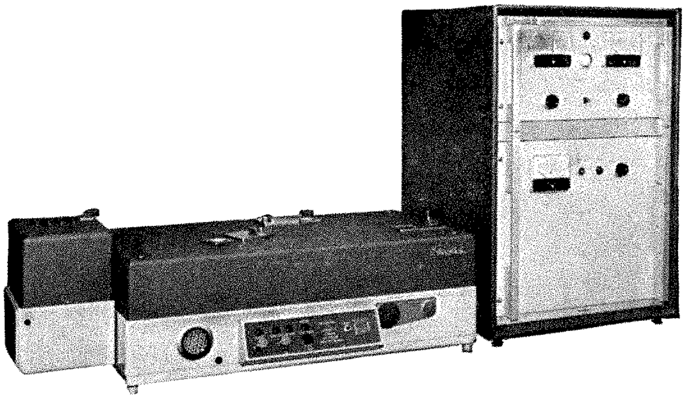

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NATURE Volume 226
APRIL 18, 1970

Long Shadow from Apollo

By now, the daily newspapers will tell how the saga of Apollo 13 has been finished. Whatever the outcome, the successful firing of a rocket engine in the early hours of Wednesday (GMT) seemed at the time a good augury only against the hazardous background of potential catastrophe. To be sure, it was lucky that the trouble with the oxygen tanks in the command module did not happen in still more alarming circumstances, after the fuel in the landing vehicle had been used up, for example. That would have been worse. As things turned out, it is also inevitable that there should have been heart searching about the events preceding the launching less than a week ago—the trouble with the fuel tanks and the sudden replacement of one pilot. None of this can conceal, however, the great courage with which those directly involved carried on with their work. Even if Apollo 13 missed the landing on the lunar highlands, it provided a moving and more public demonstration than ever of how people can face the prospect of sudden death in a seemly and modest fashion.

One of the striking features of the past days has been the sheer competence of the people who have been concerned, on the ground as well as in the sky. As always, NASA has put on a good show, and there is more to this than the mere capacity to keep a stiff upper lip or even to diminish real dangers. Of all the claims which have been made in the past several years that the exploration of space has brought unexpected benefits in unrelated fields, perhaps the most important is the least tangible—the way in which the organization functioned superbly in the course of the most daring exploits. A part of the secret has been that there have been enough men on hand to think of everything, or nearly everything. Another has been the logic of the planning. By themselves, even armies of design engineers could not have accomplished what the Apollo project has done in less than two years—since Mr James Webb unaccountably gave up his job as administrator, the hydrogen rockets of the second and third stages have been successfully brought into service, and the whole system of hardware for landing on the Moon has been tested and used to its full capacity on two occasions. For as long as people are concerned to do things for the first time, the conduct of Apollo will be a monument to the way in which pure reason can accelerate complicated technical developments. Nothing decided in the next few months should diminish the importance of this brilliant demonstration. Those who are inclined to complain that there has been an accident should, rather, wonder why it has not happened before.

What will happen next, in the next few months and in the decade which lies ahead ? The immediate future is in no doubt. It is unthinkable that the flight of Apollo 14, originally planned for the end of October, should continue without the kind of careful analysis of the past few days which followed the death of three astronauts by fire just three years ago.

Whatever the examination may reveal—and NASA has a good reputation for honest self-criticism—the outcome is likely to be a further attenuated sequence of journeys to the Moon. Most probably it will be 1971 before the next Saturn V rocket leaves the ground. Two things should be said. First, it is important that two journeys to the Moon have been successful, that the trouble with Apollo 13 has probably been the result of some statistical error and that there is no reason why the same equipment should not function well on some other occasion. But the way in which the great mass of material returned by Apollo 12 is still being filtered through the terrestrial laboratories should also be a comforting sign that there is no great need for hurry. If the purpose of these visits is to bring back a better understanding of the Moon, no serious damage will be done if the interval between successive flights is more like a year than the six months now allowed for.

The accident has come at an awkward time for NASA, with the budget for the year to the middle of 1971 still lying in the lap of Congress. Suffused with the confidence of two successful landings on the Moon, NASA has turned its attention to other projects—the space station in orbit about the Earth (with all kinds of astronomical telescopes to give it point) and the scheme for making a re-usable rocket or shuttle. The space station would take a good part of the effort in the year ahead, but the shuttle would remain more or less on the drawing boards—indeed, only the design of the engine would be carried this year to the point at which contracts could be placed. All this is part of the Nixon administration's strategy for the next decade—a somewhat humdrum preoccupation with two new aspects of the technology of space flight and some spectacular frosting on the cake to round it off—the grand tour of the outer planets on which automatic rockets could be sent in the later seventies. In the past few months, and with the success of the last two journeys to the Moon very much in mind, NASA has been pretending that possible grandeur has been postponed for the sake of a balanced budget. Now there may be a more sensitive appreciation of the staggering innovations of principle there will be in the design and development of the shuttle rocket. Is it really on the cards that such a device could be in service by the late seventies, and if so, what would it be for ? This is the kind of question likely now to be asked in Congress. Maybe NASA has an answer, but it ought to be better and more interesting than those given so far.

This may also be an occasion for yet another reconsideration of the objectives of the space programme, in the United States, the Soviet Union and the other nations from which rockets are being aimed at orbits around the Earth. It is no disrespect to the three brave men in Apollo 13 to recall that human intervention is still comparatively unproductive. The great skill which NASA has embodied in the Apollo programme is premature. Now that the technique has been learned, it would be foolish deliberately to forget it, which means that space flight should be continued on some sensible scale. Within the kind of budget to which NASA is becoming accustomed, however, there are much more urgent tasks than this. One of the disappointments of the new budget has been the postponement of some of the Earth satellites that could demonstrate the feasibility of useful arts—still better telecommunications and aircraft navigation chief among them. And where scientific exploration is concerned, it is still regrettably fair to say that the money thus spent is allocated not by the test of what will be best for science but with the extra and artificial constraint that rockets must somehow be involved. What NASA needs is a set of tangible goals in space technology and exploration, and a more open-ended programme of space travel.

The truth is that there is no great need for hurry. In the late fifties, after the Sputniks, there may have seemed good reason for an American demonstration of competence. This has now been done, and the seventies are, in any case, likely to be very different from the fifties. In a funny way, the United States space programme has become old-fashioned. There is no reason why it should be abandoned—indeed, the greatest danger now is that indifference or disillusion may make it hard to recruit enthusiasm (and money) for jobs that need badly to be done. There are also splendid opportunities, as Dr Thomas Paine is always saying, for making space research much more a vehicle for novel forms of international collaboration. But in circumstances like these, over-definite objectives such as the space station and the shuttle may be less valuable even as spurs to space travel than a more open-ended programme, with one step following deliberately on another. No harm would be done in making fuller use of the Saturn rocket before going on to fresh imponderables.

Passivity and Activism for Stockholders

SEVERAL universities in the United States are being assailed by students and others moving under the label "Campaign GM" to make a radical reappraisal of their attitudes towards their investments. GM means General Motors, one of the stocks widely favoured by those anxious to see that academic endowments are not too dangerously dependent on the ups and downs of the stock market. The objective of the student campaign is to persuade universities holding large amounts of General Motors stock to play a more active part than has been their custom until now in the management of the company and, in particular, to set up a committee of inquiry to assess the vigour of the corporation's attempts to produce cars which are safe and free from pollution, to add three directors to the board "who will insist that the board takes account of the many social consequences of its decisions" and also to amend the corporation's charter so as to prevent it "from engaging in conduct which is illegal or detrimental to the public health, safety and welfare". The campaign has already picked out the three extra directors it would like to appoint—one of them is Dr Rene Dubos of the Rockefeller University. Understandably enough, the management of General Motors plans to resist these proposals at the shareholders meeting on May 22, chiefly on the grounds that they are tantamount to intolerable restrictions on the management of the company. University administrators are finding it less easy to make up their minds, not least of all because it seems plain that a decision to intervene in the management of General Motors is likely to imply that more interest will have to be taken in other companies and their affairs. The fact that the University of Pennsylvania was the first of several universities now under pressure to agree that its 29,000 shares should be voted in the sense of the campaign's proposals is unlikely by itself to carry weight elsewhere, and it is only fair to expect that even if the university stockholders are unanimous, their total holding of rather less than a half per cent of General Motors stock will not be decisive. Nevertheless, the issues that have been raised are important and also interesting.

The first reaction of universities elsewhere will be envy at the way in which so many American universities seem to be provided with endowments, embarrassing or otherwise. Others will recall that many Oxbridge colleges have had and still have investments which consist of parcels of land which are sometimes managed with less concern for social values than many academics would have wished. The way in which the Commissioners of the Church of England used to be attacked for their ownership of brothels in London was in its way a more obvious anomaly than the case of General Motors—after all, the allegations of social irresponsibility against the manufacturers of automobiles are open to dispute even if the authors of Campaign GM think their validity is self-evident. For universities, however, the question is how and when decisions about investments should be tempered by an awareness of the uses to which the investment is put. The traditional view of university treasurers has been substantially that of Dr Nathan M. Pusey, the president of Harvard University, who said some years ago that "our purpose is just to invest in places which are selfishly good for Harvard—we do not use our money for social purposes". On the strict interpretation of the terms under which many bodies of trustees must function, universities have no alternative. Many trust deeds leave no room for trustees to do other than maximize the financial return on what they are able to invest. To say that the prospect of a high yield should be forgone because the company concerned is making products which are socially undesirable—armaments perhaps—may often be a kind of dereliction of duty. To be sure, it would often be hard to make such a charge effective so that trustees might be able to meet some particular objections without running into trouble. The more practical difficulty is that of asking the managers of large and diversified endowment funds to make decisions which are acceptable to faculty and students on all new investments. If ever there were a prescription for making sure that academic productivity was completely prevented by the length of faculty meetings, this is probably the one. A further difficulty, harder to avoid, is that the instincts which have helped to launch Campaign GM are as much concerned to undermine the power of big corporations as such as to make sure that General Motors manufactures different kinds of motor cars. There is, in other words, a danger that if the universities take the wishes of Campaign GM too seriously, they may well find themselves with no alternative but to lend their money to the banks, which would, no doubt, in turn pass it on to the big corporations.

In short, Campaign GM must not be thought of as a serious attempt to redirect the financial resources of industry but as a gesture by the universities towards the large corporations. As such, and particularly because the specific proposals which have been put up for the shareholders' meeting on May 22 are unlikely to succeed, there may be some virtue in letting the issue out into the open. To begin with, it will help to give universities in the United States a more accurate appreciation of their financial strength and weakness if there is now some public discussion of the character of their investment funds. Paradoxically, Campaign GM has come at a time when a great many private universities have been forced to recognize that costs are rising much faster than their endowment income. Gestures of self sacrifice, even if they consist merely of persuading General Motors to be less profitable by being more socially responsive, will have a certain heroism. The second benefit of this confrontation should be that universities will have to think out more clearly than they have done so far the ways in which companies can be made more socially responsive. In the long run, of course, seeking as Campaign GM does to impose restraints on corporate management is by no means the most effective way in which universities might influence the course of industrial events. Where the safety of motor cars is concerned, for example, universities could do valuable work by playing a more vigorous part in the attainment of protection for consumers by the development of technical standards of performance. This kind of activity is more logically a part of the philosophy of Campaign GM than the attempt to pack the board of directors with high-minded people. But, that said, it will be interesting to see how General Motors will respond to the demands now made on it.

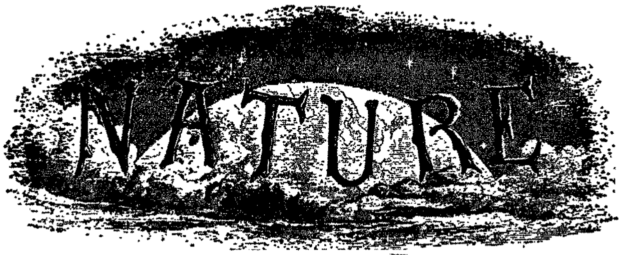
Mr Nader's Crusade

EVEN if some of Mr Ralph Nader's charges against the Food and Drug Administration are exaggerated and even biased, the report which his team has now published (page 205) deserves and will get close attention. Of the criticisms levelled at the Food and Drug Administration, the most damaging are those which argue that the administration is trying to do much with a staff which is too small and ill-provided. The more sensational parts of the report, such as that which retells one version of the story of how cyclamates came to be banned from the market in October last, are less reliable. Even now, six months later, it is hard to know who is right and who is wrong about cyclamates. Some evidence of the causation of bladder cancer by cyclamates has accumulated in studies with laboratory animals, but there is no knowing how the extrapolation from animals to human beings is to be accomplished. At the same time, the usefulness of cyclamates as means of sweetening food and drink without making people fat has been called into question and plainly further study would be necessary if, in the present climate of opinion, cyclamates were being introduced for the first time into foodstuffs. Mr Nader's team, in its criticism of the FDA, tends unfortunately to paint too sharp a picture of the disagreements between officials within the organization. It may be true that some officials had been pointing out for several years that cyclamates might be harmful, but it does not follow from anything that Mr Nader has said that the FDA was wrong in overriding this advice. Indeed, the most telling point of his team's criticism of the FDA on cyclamates is that in the end the ban was imposed on the narrow grounds provided by the Delaney amendment. Plainly it would have been better if the case for prohibition could have been more broadly based. The trouble is that then, as now, the case is not yet convincing.

The case history of the cyclamate ban is important, however, because it calls in question the capacity of the Food and Drug Administration to act vigilantly and quickly in the regulation of foodstuffs. The truth is that to provide a cast-iron demonstration that some substance, either a foodstuff or an additive, can be marketed safely is bound to be time consuming and expensive. Mr Nader's team assumes that the FDA should be able to carry out all the investigation needed to justify the inclusion of new materials in the safe list. There may be a case for holding this point of view, but if it were accepted, it would be necessary to increase the research strength of the FDA not merely by a factor of two or three but by an order of magnitude.

This is why one of the most disappointing features of the new report, the most recent in Mr Nader's programme to expose all the regulatory agencies to scrutiny, is that it has very little to say about the philosophy which should be adopted by organizations like this. In the past twenty years, the Food and Drug Administration has made a good name for itself by developing and enforcing standards of purity for food. More recently, it has plainly found it harder to keep up with the monitoring of innovation. The ambitious but well intended plan to drive off the pharmacy shelves not merely drugs which are dangerous but those which are in some sense or another unnecessary is having to be farmed out to committees such as that organized by the National Research Council, a procedure which exposes both parties to the bargain to some unnecessary hazards. The difficulty is that any other course would require a Food and Drug Administration which is not merely able to keep pace with the manufacturers but which can also anticipate their innovations.

100 Years Ago



The distance across the Channel on the line chosen is about 22 miles. The tube as proposed is large enough for the passage of carriages of the present ordinary construction, and to avoid the objections to the use of locomotives in a tube of so great a length, and the nuisance which would be thereby created, and taking advantage of the perfect circular form which the mechanical operation of turning, facing, &c., will insure, it is proposed to work the traffic by pneumatic pressure. The air will be exhausted on one side of the train and forced in on the other, and so the required difference of pressure will be given for carrying the train through at any determined speed. Powerful steam-engines, with the necessary apparatus for exhausting and forcing the air into the tube, will be erected on shore at each end; and supposing one tube only to exist, the traffic will be worked alternately in each direction.

It has been found by calculation, that, for moving a large amount of tonnage and a great number of passengers, the most economical arrangement will be to send combined goods and passenger trains through the tube at 20 miles an hour, with occasional express trains at 30 miles an hour. Thus, an ordinary or slow train would occupy about 66 minutes in the transit, and a quick or express train about 45 minutes. In this way the tube, if fully worked, would permit the passage of 16 ordinary slow trains (8 each way), and 6 express trains (3 each way), each conveying both goods and passengers. About 10,000 tons of goods per day, or upwards of 3,000,000 per annum, and 5,000 passengers, or nearly 2,000,000 per annum, might be taken through, or a less amount of goods and a larger number of passengers, or *vice versâ*, if circumstances rendered other proportions necessary or desirable.

From a report in Nature, 1, 632, *April* 21, 1870, *of an Anglo-French scheme for constructing a Channel Tunnel.*

OLD WORLD

SALARIES

Pay Rise for Dons

by our Education Correspondent

THE latest recommendations of Mr Aubrey Jones's Prices and Incomes Board on university teachers' pay are unlikely to provoke the howls of indignation and the resignations that greeted the first report. An across-the-board rise of 9 per cent, backdated to October 1, 1969, has been recommended (*Standing Reference on the Pay of University Teachers*, Cmnd 4334, HMSO, 6*s*). It is true that this goes little more than half-way towards meeting the claim put in by the Association of University Teachers last December, but at least the stumbling block of the previous report—that students should have some say in assessing the teaching ability of staff—has been tactfully dropped.

The award will add about £9 million to the total salary bill, and will give assistant lecturers an increase of about £112. The average professor will find himself better off to the tune of about £420 a year. The Association of University Teachers (AUT) asked for increases ranging from 11 per cent for junior lecturers to 15 per cent for professors, and Mr Laurie Sapper, the AUT's general secretary, said that although the association is disappointed that its claim has not been met in full, it is only an interim award and the offer which will be made in October this year may be nearer the level asked for.

When the AUT put in its claim last December, it argued for greater increases at the top end than at the bottom of the salary scale, because shortage of finance has reduced the ratio of senior to junior teachers and caused career prospects to deteriorate. This argument cut no ice with Mr Jones's committee, which suggests in its report that career prospects would be improved much more by removing the UGC's fixed ratio of senior teachers to total number of staff. This proposal, which is supported by the AUT, seems to have been left out of the list of recommendations given at the end of the report, and even if it is accepted by the government it may not make any immediate difference to staff ratios. In most departments, except medicine, the proportion of senior staff is in any case well below the figure recommended by the UGC.

University teachers have always been bitterly opposed to government regulation of their salaries through Mr Jones's committee, and Mr Sapper said that the AUT "rejects completely" the proposal that the Prices and Incomes Board should be involved in further negotiations about university salaries. New machinery for negotiating salaries, which involves direct talks between the AUT and university authorities, is being studied by the Secretary of State for Education at the moment, but the Prices and Incomes Board report suggests that such machinery should not be brought into operation until 1971. A study of university teachers' pay commissioned by the Prices and Incomes Board, and being carried out by the Higher Education Research Unit at the London School of Economics, should be completed in March 1971, and until that time Mr Jones's committee wants to stay in the saddle. But at least British dons are not alone in their opposition to current methods of salary negotiation. Australian research scientists were recently awarded salary increases of as little as 3·5 per cent by the Commonwealth Arbitration Commission (*Nature*, **226**, 99; 1970), a decision which sparked off widespread criticism of the arbitration system and prompted the scientists to commission a job evaluation survey by a firm of management consultants. Lower grades of research scientists in Australian laboratories earn a minimum of about £2,900, compared with £1,240 for assistant lecturers in Britain.

COMPUTER POLICY

Hard Sell for Software

AN amazing case of duplication of effort in government departments was described at last week's hearing of the Select Committee on Science and Technology investigation into the British computer industry. Mr Kenneth Barnes, managing director of Systems Programming Ltd, and self-appointed spokesman for Britain's £10 million software industry, alleged that the software for each of twenty-three payroll systems for use on ICL 1900 series machines in government departments was developed separately, a revelation which Mr Eric Lubbock described as "shocking". According to Mr Barnes, the matter is already attracting the attention of the Civil Service Department, but there is no mechanism in government departments to make known the programs which have been developed for 1900 series machines.

At present much of the software work for government departments is carried out internally, although the Civil Service Department seems to be reassessing this policy. Naturally, Mr Barnes wanted to see more of the software work put out to tender, and this view was supported by Mr Saul Steinberg, the controversial chairman of Leasco Data Processing Equipment Corporation, who was the chief witness at this week's hearing. Citing experience in the United States, where there is almost no software work carried out internally by government departments, Mr Steinberg said that the competition for contracts is good both for government and for the software industry. In the United States, Mr Steinberg said, secrecy is no obstacle and even the most sensitive contracts in national defence are given to private companies.

Pressed to give his opinion of the prospects for International Computers Ltd, the British computer manufacturer, Mr Steinberg said that ICL is in difficulties because it is marketing two unlike computer systems —System 4 and the 1900 series. This is something which even IBM would certainly never undertake. In any case, the British market is too small; a market the size of Europe is the minimum for a manufacturer such as ICL. But chauvinistic feelings are bound to make impossible the strategy of merging what is left of the European computer industry in one move. What ICL should do to break into foreign markets is merge with one international company, and build up from there. The government could help by giving ICL a contract for the development of machines compatible with both System 4 and the 1900 series, if that were a feasible task to attempt.

But both hearings concentrated on the British software industry, which Mr Barnes expects to grow by at least 30 per cent per annum up to 1974. The invasion of the British software market which Mr Barnes was prophesying two years ago has not come about to the extent which was feared, Mr Barnes said, but nevertheless it is crucial that the British software industry take action to build up its muscles. Compared with the largest American software houses with up to 3,000 employees, British firms are small fry with staffs of a few hundred which are not equipped to tackle large-scale systems. The industry must take steps to consolidate itself, but mergers will be difficult to achieve bearing in mind that the chief assets of the companies are their staffs of independently minded individuals. According to Mr Barnes, the Industrial Reorganization Corporation has been considering a merger of three software houses, but it was obvious that nothing would come of the idea. One approach, Mr Barnes agreed, would be to merge software houses with management consultants. This should produce organizations capable of integrating computer systems with management more effectively—both witnesses recognized that this is an important aspect of software work which needs more attention.

COMPUTERS

German Companies Join Forces

THE proposed merger of the computing interests of Siemens and AEG-Telefunken, the two West German companies, is expected to bring a breath of fresh air to the discussions on a giant European computer which have been labouring somewhat during the past months. Although Siemens is already the second largest European owned company in Europe, after International Computers Ltd, the benefits of a more unified West German approach to European collaboration are felt to be more than adequate compensation to other European computer firms for any increased rivalry that may accrue from the proposed merger.

The West German Government has been stepping up dramatically its investment in the national computer industry, partly to counter growing pressures from American companies. Government aid is 90 per cent up this year compared with 1969, and is expected to reach £34 million over a five-year period. The importance attached to the industry became clear when Dr Stoltenberg, the Minister of Science in Dr Kiesinger's government, created a new department to coordinate government activity in this area.

The chief impact of the proposed merger will be in the large computer market, in which AEG-Telefunken is strongest.

There is little indication of any intention to merge the interests of the two companies in smaller computers, which may help to account for the cheerful view of ICL, whose specialities lie outside the large computer range. But ICL is naturally aware of the inroads an expanding German industry could make into its own buoyant markets in Eastern Europe and France.

Technical incompatibility between the machines of ICL and Siemens has been held by some West German sources to be partly responsible for dulling the talks on the Aigrain proposals for a giant European computer. But there is renewed hope now that the European talks will gain some new momentum. Certainly the West German company will feel itself in a stronger negotiating position.

TECHNOLOGY

Cold Line Diplomacy

"TECHNOLOGY has now abolished 'foreign affairs'," remarked Mr Wedgwood Benn, on the eve of his 10-day official visit (April 14–24) to the United States. The volume and complexity of material to be exchanged between countries today cannot be squeezed through such a narrow pipeline—like pouring water through a bootlace, as has been said in a similar context. The traditional procedures of communication between governments are very cumbersome. "At present it takes three months to write a letter to a minister of another country—and three months to get an answer back. What we want today is not hot lines but more cold lines. . . ."

In pursuit of this idea the Minister of Technology has proposed an informal bi-monthly lunch club for Euro-technology ministers, alternately meeting in Paris, Bonn and London. Last week's London visit by M. Ortoli, the French science minister, and Germany's deputy research minister, Herr von Dohnanyi (at the British minister's invitation), was something of a trial run. They were here to coordinate Europe's preliminary response to NASA's recent invitation to participate in the American space programme for the 70s (see *Nature*, **226**, 2; 1970). Mr Benn does not believe Europe can afford to delay declaring an interest until the midsummer European Space Conference. On the other hand, further technical and financial studies of the proposal are still needed. Perhaps Mr Benn on his present visit can set up a cold line between Millbank and the office of NASA's chief, Dr T. Paine.

POLLUTION

Less Oil on Troubled Waters

STERNER government measures to restrict the discharge of persistent oils into the sea can be expected soon. The Board of Trade announced last week that a bill to be introduced into Parliament as soon as possible will greatly extend existing restrictions, and will include an absolute ban on the discharge of any persistent oils —crude, fuel, heavy diesel and lubricating oils—from the cargo spaces of tankers within fifty miles of land. This will reinforce the present ban on the discharge of all types of oil within United Kingdom territorial waters, which usually means three miles from land.

The bill is designed to modify the Oil in Navigable Waters Acts of 1955 and 1963, which are being brought into line with the International Convention for the Prevention of Pollution of the Sea by Oil as amended in October 1969. This convention was first agreed in 1954 by the member countries of the Assembly of the Inter-Governmental Maritime Consultative Organization (IMCO), a specialized agency of the United Nations. The latest amendment prohibits the discharge of persistent oils into the whole of the North Sea, the English Channel, and more than 1,600 miles westward across the Atlantic Ocean and south to Finisterre. Furthermore, new ships of 20,000 gross registered tons or more may not discharge these oils anywhere at sea. Britain is one of the first countries to take action on this amendment, which cannot come into force until it has been adopted by two-thirds of the contracting governments. The convention is now in force as it was amended in 1962; in that form it has been accepted by forty-one countries.

The full scope of the legislation to be introduced in Britain will not be known until the bill is published, but there will be measures to facilitate enforcement of the proposed ban by requiring tankers to keep more detailed records of cargo movements and disposal of oil residues. The fines imposed for unlawful discharge of oil within British waters and by British ships in other waters are also under review, and tougher penalties could form part of the new legislation. At present the fine is £1,000 on summary conviction, with no limit on fines imposed if conviction is on indictment.

The Board of Trade reports that last year there were eighty prosecutions for unlawful discharges of oil, resulting in seventy-seven convictions, compared with sixty-two in 1968 and fifty-nine in 1967. Three of the seventy-seven of last year's convictions were for discharges outside harbours, and the rest for offences within the jurisdiction of harbour authorities. Fines imposed totalled £17,550, from which five awards amounting to £792 were made towards the cost of removing the oil pollution.

ENVIRONMENT

Not Just Talk

CONSERVATION is now catching on fast enough for good advice to be out of date before it is given. The Countryside in 1970 Committee has unwittingly confirmed the encouraging growth of activity by publishing this week a report on conservation policy whose proposals for administrative reform have mostly been put into practice already. Its chief recommendation, that responsibility for the environment should be centralized in the ministers who deal with local government and planning, has even fallen short of the government's action in creating a single Ministry of Local Government and Regional Planning under Mr Anthony Crosland, which occurred after an *ad hoc* group had prepared the report (*Governmental Responsibility for the Environment*, available from 19 Belgrave Square, London SW1).

The group, under the chairmanship of Lord Hurcomb and including Sir John Winnifrith, Mr Max Nicholson and Mr Walter Lane, also recommends that the relevant ministers appoint a conservation adviser, a demand that has been at least partly satisfied by a central scientific unit set up by Mr Crosland to look after the government's pollution research. The unit is headed by Dr Martin Holdgate, whose status is at the level desired by the Countryside in 1970 Committee, for he will be directly responsible to Mr Crosland and his unit will work closely with the government's chief scientific adviser, Sir Solly Zuckerman. A further bonus that the committee did not call for is the Royal Commission on pollution that was announced at the same time as the scientific unit.

Among the voluntary bodies dealing with conservation, the report says, there may be a confusing degree of overlap, but the coordination that it wanted has arrived in the shape of the joint Committee for Environmental Conservation, which was being planned while Lord Hurcomb's group was working. This committee is now bringing the voluntary associations together in studies of pollution, road transport and coastal management, and also intends to carry out the second function wished on it by the report of confronting the government with recommendations when it feels a collective voice would be useful.

Some of the advice of the *ad hoc* group remains to be taken, notably at a local level, where it would like to see more strength given to the County Countryside Committees that were created, without legislative backing, as a result of the second Countryside in 1970 Conference in 1965. These groups, the report argues, are performing splendidly by providing a focus for environmental responsibility that would otherwise be dispersed among too many organs of local government, and there should be more of them. Because the future of local government is not clear, while the Redcliffe-Maud reforms are being pondered, the report makes no specific demands of local authorities but urges that, when they are reorganized, they should be made to "have regard to the desirability of conserving the natural beauty and amenity of the countryside", as was laid down by the 1968 Countryside Act. Elsewhere, however, the Countryside in 1970 Committee will have little to do but wait and see how far its ideas are followed in the spirit as well as the letter.

LIBRARIES

Bloomsbury for Books

by our Planning Correspondent

THE announcement in the House of Commons last week by Mr Short, Secretary of State for Education and Science, that the British Museum Library is, after all, likely to be rehoused on a site facing its present one in Bloomsbury has been welcomed all round, and particularly by the museum trustees who through years of frustration have stoically maintained that the area on the south side of Great Russell Street was the only practical site worth expanding onto. Mr Short also said that the government has accepted in principle the chief recommendation of the Dainton report on National Libraries, published last year, that a national libraries authority should be established to administer the museum library, the National Reference Library of Science and Invention (at present part of the British Museum Library), the National Lending Library, the National Central Library, and the British National Bibliography. The government, he said, was considering the structure and responsibilities of such an organization and it plans to issue a White Paper after it has had further consultations.

The suitability of the Bloomsbury site was first officially established in 1962 when the then Minister of Public Building and Works, Mr Geoffrey Rippon, appointed architects to prepare a design. The site was also favoured by the Dainton Committee, in spite of an earlier rejection of it by the government in 1967 on the grounds that it would have meant the demolition of a large number of houses in an area with a housing shortage. Mr Short now says, however, that it may be possible to rebuild the museum library and house the National Reference Library of Science and Invention as well, on a much smaller site than was originally planned because of recent technical developments in the storage of books and other library material. This would mean that fewer houses would have to be pulled down, and the whole of the west side of Bloomsbury Square would be preserved. This decision should help to calm both the preservationists and the Camden Borough Council, who have been the most vehement opponents of the original scheme to rehouse the museum library.

An important implication of the government's change of mind, and one that all scientists and librarians will welcome, is that it puts the future of the present National Reference Library on a more secure footing. Denied the site on the South Bank, and with its valuable stock scattered over London in several places, the library cannot offer the public the same level of service as if it were functioning in one central building and constituted, with the British Museum library proper, a National Reference Library in every sense. An added advantage of the Bloomsbury site for such a library is its proximity to the Patent Office, and to the University and other scholarly library collections in central London.

BRITISH MUSEUM

Falling Star Cast

MOON dust may have its devotees and tektites their fanciers, but neither form of extraterrestrial matter can compete with the fascination of meteorites. Last week the British Museum (Natural History) opened a new exhibition which at last does justice to what must be the most comprehensive collection of cosmic debris in the world.

Dominating the new pavilion like the Kaaba at Mecca is the squat, 3½ ton mass of the Cranbourne meteorite, coddled in an atmosphere of dry nitrogen lest its lawrencite corrode to rust. Illuminated glass-fronted niches on either side of the darkened chamber hold examples of the irons, stones and stony–iron meteorites. In a well at the centre lies a model of the swarm of meteorite craters at Henbury in Central Australia. The elegant design and exposition of the pavilion stand in sharp contrast to the faded Victorian glories of the mineral gallery whose long aisle the visitor has to walk to reach it.

The museum's collection began with a fragment of the meteorite which was discovered by P. S. Pallas in Krasnojarsk in 1775 and presented by the Academy of Sciences at St Petersburg. Since then the museum has acquired specimens from some 1,215 of the 2,127 known falls. The museum's catalogue of meteorites falls, the third edition of which has recently been completed by Dr Max H. Hey, is recognized on an international basis and serves, among other things, in directing people who require samples of particular falls to the museums that have most to spare. For those who have despaired of going to catch a falling star, the meteorite pavilion at least provides the consolation of being able to stand surrounded by part of the leftovers of the primitive solar system.

GOVERNMENT RESEARCH

Will BRDC Work?

THE latest attack on the green paper on industrial research and development in government laboratories has come from the Institution of Professional Civil Servants, which represents many of the staff in the organizations likely to be affected by the proposals if they go through. At the same time the Minister of Technology, Mr Anthony Wedgwood Benn, from whose department the proposals originated, has been defending the green paper to his critics on the House of Commons Select Committee on Science and Technology.

Much of the criticism is that the green paper is not sufficiently specific in its outline of the plan to form a British Research and Development Corporation out of what is left of the Atomic Energy Authority, the research and development laboratories of the Ministry of Technology which are undertaking civil work, and the National Research Development Corporation. This lack of detail is something which Mr Benn said was deliberate, to provoke discussion of the green paper, and he said that some of the proposals will be modified in the light of what has already been said.

But the essence of the IPCS criticism is the timescale which is envisaged for the setting up of BRDC. By 1975 it is intended that the corporation be able to support itself, earning one-third of its income from industry and the remainder from government sources. On this point the deputy general secretary of IPCS, Mr John Lyons, says that if the government sticks to the five-year period for generating one-third of the income of BRDC, this is "tantamount to an intention to create redundancy by taking away the income—if that is the case we will strenuously oppose the BRDC concept". The IPCS attitude is that a target contribution from industry of 15 to 20 per cent over a much longer period would be more realistic.

Mr Benn's approach to this point at the meeting of the Select Committee is that the target contribution from industry is not likely to be as difficult to achieve as people imagine. Although the gross annual cost of the organizations which would make up BRDC is currently estimated at £70 million, Mr Benn said that by 1975 this figure will be down to £60 million. The organizations already earn £10 million from industry, including £1 million for irradiation services in the Dounreay reactor and £1·8 million for the sale of electricity from prototype reactors. But if people fail to attract contract work from industry, Mr Benn said, then he would have to ask whether these people should be kept on. Despite this explanation, however, it is clear that the estimate of the industrial contribution to the running of BRDC is a major stumbling block to acceptance of the proposals. Many people would like to have seen a market survey, but this was ruled out by Mr Benn on the grounds that a market survey is something which is carried out when one is starting from scratch, whereas in this case he is dealing with established organizations.

- Mr Lyons of IPCS also criticizes the assumption that the Atomic Energy Authority has now fulfilled its purposes, a tenet that is taken as one of the justifications for BRDC.

IPCS believes that the directors of the organizations in BRDC should be free to develop their establishments within the framework of general policy, that moves to introduce a split between civil and defence work should be resisted, and that the finances of BRDC should be worked out on a long-term basis, preferably five years ahead or more.

Whether or not the IPCS supports the proposals in the green paper, the institution says, depends on the attitude of Mr Benn to these questions. For the moment, it reserves its decision.

Parliament in Britain

Euthanasia

THE campaign to legalize euthanasia seems to have met its end. The latest attempt was cut short when Dr Hugh Gray failed to bring in his Voluntary Euthanasia Bill. Dr Gray's bill sought to give every individual the choice between life and death if he ever became the victim of an incurable illness. The choice at the moment, he said, rests with the doctor, and although many would not seek to prolong life in hopeless cases there are some to whom life has an absolute value and who believe that it should not be taken away even when the patient is reduced to a living organic shell. Dr Gray argued that such a situation infringes individual liberty. The bill sought to give legal status to a declaration, either for or against euthanasia, which every individual could sign and which would be kept with his medical records.

Mr Norman St John-Stevas, who opposed the application to introduce the bill, pointed out that its intentions would contravene the moral and religious code that guides the majority of people in the Christian world, because it would allow one person to kill another. A doctor, he said, is under a duty to preserve life, but that does not necessarily mean that he must prolong the act of dying.

The application to bring in the bill was defeated overwhelmingly—MPs did not even bother to vote, but just shouted "No". Four euthanasia bills have now come before Parliament, and all have been heavily defeated. (Debate, April 7.)

Higher Education

THE growing interest among MPs in higher education was in evidence last week when Mr Short and his junior ministers answered questions. The much-discussed green paper on education in the 1970s, which may partly be responsible for the interest, should be published in the early summer, Mr Short announced, and it seems that many of his colleagues are expecting it to appear in May.

One of the main considerations is the number of places in higher education which will be available in 1980, but when asked by Mr Christopher Ward whether it is government policy to provide university places for 60 per cent of those qualified in the late 1970s, Mr Gerald Fowler refused to be drawn. That, he said, is one of the matters that will have to be decided in the course of this year. One point to emerge from a later question is that the government has no intention of establishing comprehensive universities, which would incorporate all institutions of higher education. When asked by Mr Boyd-Carpenter whether the government intends to continue selecting students by ability and aptitude at the age of 18, however, Mr Short replied that he hopes that universities will become increasingly comprehensive both in the structure of the student body and in the content of the courses, providing both degree-level and other courses. But such changes cannot be imposed from the outside. The notion of comprehensive universities has been widely canvassed both by the National Union of Students and by the Association of University Teachers.

Mr Fowler also announced that teachers, employers and other interested bodies are being consulted on the possibility of raising standards of entry to colleges of education. (Written and oral answers, April 9.)

NEW WORLD

Mr Ralph Nader and Pure Food

MR RALPH NADER, the scourge of Detroit, last week turned his attention to the Food and Drug Administration. His organization, the Center for Study of Responsive Law, published the draft of a book by James S. Turner called *The Chemical Feast* (available in mimeograph at $10) which seems well calculated to keep the new Commissioner of the Food and Drug Administration, Dr Charles C. Edwards, awake at night and, at the least, to burn the ears of Dr Herbert L. Ley, the commissioner who left his job last December. Mr Nader's introduction to the report, which is necessarily as objective as its thorough documentation would imply, directs mordant scorn not merely at the Food and Drug Administration for its "failure to ensure safe, pure and nutritious food in the world's largest bread basket" but also at the food manufacturing industry "for manipulating the content of food products as dictated by corporate greed and irresponsibility". There are nevertheless enough well directed charges in Mr Nader's document to stir a pot which is already boiling vigorously enough.

The tenor of the new study is embodied in the view that "making food appear what it is not is an integral part of the $100 billion food industry. The deception ranges from the surface packaging to the integrity of the food products' quality to the very shaping of food tastes". Techniques, Mr Nader says, amount to "nutritional deception" as in the way that "enriched white flour" is flour from which most nutrients have been removed, and the way in which additives of various kinds conceal "the rapid increase of fat content in frankfurters, their decrease in meat protein and the substandard quality of the meat". One result, he says, is that nutritional standards in the United States are declining, but the Food and Drug Administration, which should be in charge, has "failed to close out the options for corporate cost reduction and camouflage" and has in the process become "an official sponsor of processing and marketing practices that transformed the defrauding of consumers into a competitive advantage".

Mr Turner's text fills out this theme. He points out that "there has been almost no increase in the life expectancy of Americans" since the beginning of the sixties and that "black American men actually die younger than they did in 1959". The United States is no longer best in the infant mortality league table, while a study carried out by the *Journal of Nutrition Education* and reported last year (*J. Nutrit. Ed.*, **1**, 39; 1969) had shown that "dietary habits of the American public have become worse, especially since 1960". But the FDA has found "its meagre but well intended efforts" stifled by the interests of the food industry and is unable to "exert any meaningful influence on behalf of the food consuming public".

The Chemical Feast begins with cyclamates and has a number of novel charges to make, chiefly on the basis of internal memoranda of the FDA. According to Mr Turner, the FDA's first official dealings with cyclamates were in January 1950, when it allowed the Abbott Laboratories to market cyclamates as tablets for use by diabetics and others even though one of its officials considered the supporting evidence to be "an illustration of how an experiment should not be conducted". The official, said to have been Dr A. J. Lehman, asked that Abbott Laboratories should be told that "it is on the basis of our own work that recommendations are being made to permit this application to become effective". The Nader document goes on to say that the data on which the FDA relied in 1950 showed abnormally high incidences of cancer among the experimental animals and that "combining these suggestions of cancer with the shoddiness of Abbott's tests should have clearly indicated that more tests of cyclamates were needed" but that "this was the first of many danger signals about cyclamates which the FDA ignored".

The new report goes on to say that further warnings about cyclamates came from the Food Nutrition Board of the National Academy of Sciences in 1954, 1955, 1962 and 1968, and quotes from the statement in 1962 the view that considerations of public welfare preclude "the uncontrolled distribution of foodstuffs containing cyclamates". Mr Nader's team goes on to complain that the FDA never "effectively circulated" recommendations about the maximum daily intake of cyclamates put forward by the National Academy of Sciences' committee in November 1968 and goes on to say that the FDA put cyclamates on the list of food additives "generally recognized as safe" without adequate testing, but with the result that their use by the public could not afterwards be controlled. One of the charges against the FDA is that it has no systematic procedure for assessing the safety of compounds once these have been included on the GRAS list. The report also complains that the FDA did not re-examine its information about substances on this list after the amendment in 1958 to the Food, Drug and Cosmetic Act (the Delaney amendment) which requires that any additive should be held unsafe if it can be shown to cause cancer in men or animals.

The report goes on to say that the FDA ignored "scientific evidence indicating the dangers of cyclamates", as, for example, the suggestion that cyclamates are transformed into cyclohexylamine in the digestive tract of many people, the report in March 1968 by Dr Jacqueline Verrett of the FDA of a "firm relationship between cyclamates injected into chicken's eggs and deformities of embryos taken from the eggs", and the discovery, circulated within the FDA by Dr Martin Legator, that N-hydroxyl cyclohexylamine had been isolated from human prisoners given cyclamates. The study goes on to say that the commissioner of the FDA, then Dr Herbert Ley, ignored "at least ten other danger signals" in the period up to December 1968, including evidence of interference with anticoagulant therapy, unexplained liver damage, intestinal trouble and interference with drug action. The report also says that commercially available cyclamate contains "the dangerous and untested chemical cyclohexylamine" and that feeding tests had also shown that cyclamates "have limited effectiveness for weight control even under controlled dietary conditions". One of the most damaging passages in the report is the assertion that Dr Herbert Ley responded to a recitation of these points on December 12, 1968, by issuing a statement that although "totally unrestricted use of cyclamates would not be warranted", there "was little in the then widespread general use of cyclamates to be concerned about". The Nader study discounts the value of the report of the committee of the National Academy of Sciences set up specifically to study cyclamates and on which Dr Ley's public statement had been based by quoting from an internal memorandum in which two FDA officials describe that study as "a largely uncritical review of the available material".

The line of argument on cyclamates in the Nader study is that the staff of the FDA had accumulated enough evidence about the toxicity and teratology of cyclamates to justify their banning on more general grounds than those of the Delaney amendment on which cyclamates were removed from the GRAS list in October 1969. The study indeed complains that the Secretary of the Department of Health, Education and Welfare, Mr Robert A. Finch, misunderstands the Delaney amendment and its purposes, does not accept the notion that additives should be shown to be safe before being put on the market, underestimates the "growing scientific concern" about environmental chemicals and discounts "the protection of every scientist's right to communicate his findings to his fellow scientists and the general public".

The attack on the FDA includes the assertion that the narrow grounds on which cyclamates were eventually banned had meant that "the decision has easily been eroded by special interests". It would have been better, the argument goes, for Mr Finch to have asked manufacturers to demonstrate the safety of their products once doubt had been cast on them. The report says that "it was cynically irresponsible" of Mr Finch to retreat from the full scale ban, due to have become effective on February 1, 1970, by allowing first that foods containing cyclamates might be sold up until September 1, 1970, and then to suggest (on November 20, 1969) that foods (but not drinks) containing cyclamates might be sold so long as they were labelled. The Nader study goes on to suggest that the National Academy of Sciences committee on artificial sweeteners was not told of the concern within the FDA about cyclamates until 1969, that the advisory committee which drew attention on November 18, 1969, to the medical benefits of cyclamates did not consider evidence that these materials are ineffectual and that the Surgeon-General of the United States "has continued to participate in misleading the public and the scientific community" by concealing what he knew about the incidence of bladder cancer at low dosage in an article to which he contributed (*Science*, February 20, 1970). In summary, the Nader study says that the "cyclamate case establishes that the FDA has been less than diligent in applying the food protection laws. The Nader team is particularly cross that Mr Finch "never mentioned evidence that birth defects and genetic damage which were related to cyclamate in tests on laboratory animals are a more serious danger than cancer". For Mr Nader's team, the cyclamate decision must be viewed as the beginning, not the end, of public concern about chemicals in the food supply.

Although one of the most tangible parts of the attack on the FDA, the tale of the cyclamates is only a part of what the Nader team has to say. It complains, for example, that the FDA has been more anxious to root out food cranks and those who profit from them than to build up a capacity to keep a continuing watch on the practices of the food industry. It says that the FDA has cravenly capitulated to the demands by cola drink manufacturers that they should not be required to mention caffeine on their labels, complains that the FDA is wrong to insist that vitamin preparations should be labelled so as to indicate that "there is no scientific basis for recommending routine use of dietary supplements" and says that the FDA has been weak in promulgating standards for orange juice.

In general, the Nader study says, the FDA minimizes "the hazards of the chemical environment", denies the existence of under-nutrition, pays little attention to the risk of food poisoning from new methods of food manufacture and distribution and ignores research on the causes of heart disease and stroke.

Why should this be? The Nader team turns its attention to the food industry, now "moving towards monopoly". Advertising costs money as does the practice, attributed by the Nader study to the Campbell's soup manufacturers, of maintaining a collection of antique soup tureens "to counteract the bad image" produced when Andy Warhol showed his first pop art picture of a soup can. The Nader team says that the "purposeful and economically motivated pollution of baby foods by their manufacturers should dispel all beliefs in food industry self-regulation" and goes on to discuss the controversy about monosodium glutamate in baby food, in which the food industry "distorts or dismisses the warnings of responsible scientific critics against these components". The trouble, the study concludes, is that the FDA cannot distinguish between the interests of consumers and of manufacturers.

In a chapter on potentially mutagenic materials, especially pesticides, the report complains that the FDA has been arbitrary in its compilation of the GRAS list and that "more incredible even than this chemical by chemical deception practised by FDA officials was the agency's total acquiescence in industry's interpretation of the law". In practice, the report says, the FDA does not test additives when approving their safe use but relies on work done by others, often the manufacturers, and complains that it is muddled on questions such as the safe use of packaging materials.

One of the most serious charges against the FDA in the Nader document is that an internal study of the FDA's operations by Dr Maurice D. Kinslow in August 1969 was an "exercise in public relations" stimulated by the Nader study of the FDA and "designed to win public sympathy for an under-funded, under-staffed but honest regulatory agency harassed by Congress". One of the points in the Nader report which is likely to stimulate continued controversy is the charge that the FDA is scientifically less competent than it should be, and the document finds fault with the Kinslow report for minimizing the importance of scientific research within the FDA.

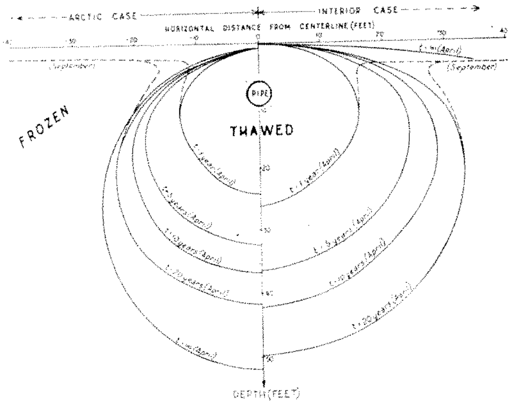
Further Delay for Alaskan Pipeline

THE difficulties of exploiting the oil fields on the North Slope of Alaska seem to be increasing. Although the scheme to build a pipeline across Alaska, between Prudhoe Bay on the North Slope, and Valdez on the Pacific coast of Alaska, should by now have been under way, it now looks as if there will be yet another public inquiry which will almost certainly mean a year's postponement. Indeed, the Conservation Subcommittee of the House of Representatives has written to the Secretary of the Interior, Mr Walter Hickel, asking for a postponement until the consequences of building the pipeline can be more accurately anticipated. At the same time, the second voyage of the oil tanker Manhattan through the North West Passage, which began last week, has coincided with the introduction of a bill into the Canadian Parliament in Ottawa that claims on behalf of the Canadian Government jurisdiction over a tract of sea running 100 nautical miles north of the Canadian Arctic coast, at least where threats to the environment (such as oil tankers) are concerned. Although the US State Department reacted smartly by saying that such an extension of maritime jurisdiction would be unacceptable, the proposed bill is likely to serve as a focus for Canadian discontent about the risk of oil pollution in the Arctic. A scheme to build a pipeline from Inuvik in the North West Territory of Canada south-east towards Edmonton, Alberta, is less far advanced than the Trans-Alaska Pipeline System (TAPS) but in the circumstances may yet be the first to be constructed. The argument about the TAPS scheme has turned on two distinct issues—questions about the rights of native Alaskans to the ownership of land in Alaska and to royalties from exploitation of petroleum reserves and questions about the damage which might be done to the environment by a pipeline of the kind proposed. The difficulties over land ownership, complex though they are, seem well on the way to solution. The federal government still owns all but a small percentage of Alaska, but when Alaska was made a state of the Union in 1958, the new state was given the right to pick for itself close on a third of the 365 million acres not otherwise allocated. But for the past two years this right has been frozen pending a settlement between the federal government, the state of Alaska and the native population, reckoned to be about 100,000, about the amount of compensation to be paid for the extinction of traditional claims to land use or ownership. It now looks as if Congress is prepared to settle for $500 million in cash, at least that amount levied as a 2 per cent royalty on oil and gas leases and close on 7 million acres of land. Since this proposal, now being worked upon by the Senate Committee on Interior and Insular Affairs, goes some way to meet the claims of the native population, the chances are that procedural difficulties will not permanently interfere with the pipeline project. In the several public inquiries about the pipeline, native Alaskans and their representatives have indeed been conspicuous by their eagerness to see the project for a pipeline go-ahead. One of the ironies of the situation is that Mr Walter Hickel, the Secretary of the Interior, was Governor of Alaska and an enthusiastic developer until he joined the Nixon Administration two years ago.

The most serious obstacle to the building of the pipeline is the serious and persisting doubt about the effects of such a gigantic engineering project on the northern terrain. The pipeline is by most standards a gigantic undertaking. The pipe itself would be 800 miles long and 48 inches in diameter. The consortium of petroleum companies which constitute TAPS (Atlantic-Richfield, British Petroleum and Humble Oil) hopes that the ultimate capacity of the line will be two million barrels of oil a day, which works out at more than 100 million tons of oil a year. This is at once a measure of the productivity of the Alaskan oil fields and a sign of how anxious the petroleum companies are to open up the North American market—it has been estimated that the interest on the sums of money so far invested on the North Slope works out at more than $200,000 a day.

So why not dig a trench and bury the pipeline in it without more fuss ? The difficulty is that much of the route runs through or over the Arctic permafrost and that the petroleum companies are more or less bound to pump hot oil through the pipe. Indeed, the petroleum is likely to emerge from the wells on the North Slope at 80° C or thereabouts, and it has been assumed that this temperature would be maintained more or less the whole way along the pipe—heat lost to the surroundings would be made good at the pumping stations, of which there would be six to begin with and twelve when the pipeline was brought to full capacity. The most obvious problem is that such a pipe would necessarily thaw the permafrost through which it ran, with profound consequences for the terrain and potential danger for the pipeline itself, for subsidence and soil movement are among the most familiar results of thawing permafrost.

The difficulties of engineering construction in permafrost have been talked of at each of the public inquiries into the pipeline project, particularly that held in Alaska by the Department of the Interior and those held in Congress by the appropriate committees of the Senate and the House of Representatives. The general feeling seems to be that the hazards of building anything, a road, a railway or a house, in permafrost country are only now being catalogued. One of the difficulties is that the stability of the permanently frozen ground a foot or more beneath the surface may depend critically on the nature of the surface cover. Removing the covering of vegetation may be enough to set in train the progressive thawing of the underlying frozen layer. Making a road surface out of a compacted layer of gravel may either melt the underlying permafrost if the gravel is a less good insulator than the



Growth of the thawed cylinder around a pipe 4 feet in diameter with its axis 8 feet beneath the surface and its temperature at 80° C. Conditions on the left represent conditions near the Arctic coast and those on the right near the southern limit of the permafrost.

vegetation which it replaces or may increase the height of the permafrost table, if the insulating effect is greater than that of the original cover, thus setting in train processes of frost heaving. Problems like these plagued the Alaska Railroad ever since it was first begun in 1910 and it seems to have been only in the past decade that the Geological Survey intervened to show how one concrete and steel bridge was being moved as much as five inches each season simply because the concrete piers, on which the steel superstructure is erected, were thermally more conducting than the gravel which they replaced.

A particular hazard in permafrost construction is the occurrence of ice wedges at places at which surface water has been able to percolate down into the subsoil and freeze. Structures like these are responsible for the polygonal appearance of the terrain in permafrost country. If engineering structures are embedded in them, or placed on top of them, catastrophic melting may occur and one of the objects of the detailed survey of the pipeline route now being carried out in Alaska is intended to identify ice wedges of this kind. It seems now to be agreed that if the pipeline project goes ahead, the owners will be required to meet the full cost of clearing up after leakages of oil caused by cracking or distortion of the pipeline. The regulations devised by the Department of the Interior, which are acknowledged even by those who resist the pipeline to be more rigorous than others to which construction companies have been subjected, also require that the operators should make good any "land harmed or damaged" during the building or operation of the pipeline, but one of the bones of contention has been that nobody is really sure how to reduce permafrost to its original condition once it has been thawed.

The scale on which the permafrost would be disturbed by a buried pipeline has been spelled out in the past few days by some calculations carried out by Arthur H. Lachenbruch of the US Geological Survey and published as Geological Survey Circular 632. It is clear that the thermal effects of a buried pipeline would become apparent comparatively slowly in a period extending over two decades or more. It seems to be agreed that there need be no mechanical damage when the content of ice in the permafrost is comparatively small. The calculations suggest, however, that a 4 foot pipeline buried 6 feet in permafrost and operating at 80° C will thaw a cylindrical region some 20 or 30 feet in diameter within a few years and that, after 20 years or so, the permafrost will be thawed to a depth of 40 or 50 feet near the southern edge of the permafrost and between 35 and 40 feet in the north, where the permafrost is colder. One of the uncertainties of this kind of calculation, based on simple assumptions about the thermoconductivity of the materials involved, is that there is no way of knowing what will happen within a thawed out cylinder of ground. One possibility is that water would collect in such a channel, causing the material within it to flow as a kind of slurry and helping to extend the region within which the permafrost is no longer frozen.

The way in which such a structure might disturb the surface drainage pattern of Alaska is one of the sources of anxiety about it. There are horror stories from Alaska which suggest that badly sited tractor tracks can permanently affect the drainage of a large tract of land. The same phenomenon would have important consequences for living things, plants and animals. One of the hazards to be thought of is that the two Alaskan herds of caribou migrate across the proposed line of the pipeline, the most spectacular of them from the foothills of the Brooks Range in northern Alaska to the Northern Slope and back again. Nobody knows what effect a strip of soggy ground 30 feet or more in width would have on the migration of the caribou, but lesser changes in the environment, the building of new roads for example, have in the past markedly changed their ways.

The hazards of burying pipes in frozen ground and the danger that a buried pipeline carrying hot oil would affect life forms in the neighbourhood is one of the reasons why there is a tendency for the planners of TAPS to think of increasing stretches of the track above ground level. Originally, it was thought that only some 15 miles out of the 800 would be above ground, principally where the permafrost is laden with ice. According to Mr George Hughes, director of the TAPS project, it is now thought that 50 to 80 miles of the pipeline will have to be above ground. Some changes have been forced on TAPS by the need for river crossings at several places on the route from north to south, and ingenious schemes for suspending some sections of the pipeline as if they were suspension bridges are being discussed. Mr Hughes also said last week that studies are now being carried out at Houston on the assumption that the oil temperature will be more like 60° C than 80° C, but the study carried out by the Geological Survey would suggest that such a change of temperature would not markedly reduce the range at which permafrost would thaw but only the speed with which the new regime was established.

Experimental studies of pipelines like this are being carried out both for TAPS by the University of Alaska, which is running hot oil through a buried section of 48 inch pipe, and by a Canadian team concerned with the planning of the Mackenzie River pipeline. The possibility that a radically different design for the pipeline would help to reduce the risk of damage has not seriously been considered and indeed, so far as TAPS is concerned, it may be a little late to think of major modifications of the scheme now that much of the pipeline manufactured in Japan has already been delivered.

OCEANOGRAPHY

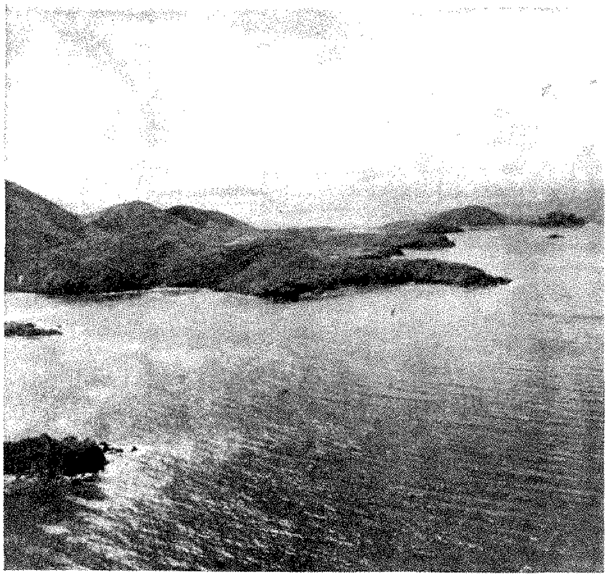
Living with Davy Jones

THE island of St John in the US Virgin Islands is now enlivened by the supporting services for an ambitious scheme for allowing fifty or more scientists of various kinds to spend intervals of two weeks or more on the sea bottom between now and the end of October. The scheme, known as Tektite 2, is based on a double-barrelled diving chamber built and operated by the General Electric Company, which has only this week staggered Wall Street with the news that it lost $42 million in the first quarter of 1970, chiefly because of strikes. This latest departure in underwater living follows a successful experiment a year ago at the same site in Great Lameshur Bay, on the south of St John. It differs from earlier sorties to the sea bottom such as the Sealab projects of the US Navy in being a means by which independent scientists can pursue diverse scientific objectives of their own. According to Dr Dail W. Brown of the Smithsonian Institution, which is spending between $50,000 and $75,000 of foundation money on the support of the scientific programme, applications from scientists to visit the sea bottom have been chosen on their scientific merits just as if the Tektite device were an oceanographic research vessel. The successful applicants for time come from universities, the independent oceanographic research institutes, government agencies such as the Geological Survey and even from private industry. But if the new submarine habitat is a unique vehicle for underwater research, it is also of practical interest to the US Navy, the National Aeronautics and Space Administration and the government of the Virgin Islands, all of which are among the sponsors of the enterprise. Also involved is the National Science Foundation, oddly enough as the sponsor of underwater spells by student technicians from three academic institutions, and the Department of the Interior, which has responsibility for mounting the enterprise as a whole and for spending something like $2 million on it.

For most of the time, the submarine laboratory, consisting of two cylinders 12 feet in diameter and 18 feet high connected by a tube 4½ feet in diameter, will be sited on a coral reef rising about 10 or 15 feet above the surrounding sea floor and at a depth of 50 feet. During the first six months, visitors will be able to spend up to eight hours a day in the water, and will be able to explore the neighbourhood of their vessels to greater depths. The crew of the vessel will consist of five people, one of them an engineer. There is also a smaller pressure vessel, with accommodation for two, which will operate towards the end of the programme at depths of up to 100 feet.

As the programme for Tektite 2 has developed, the most obvious common denominator is the opportunity provided by the new equipment for making more or less continuous observations of phenomena, physical and biological. To begin with, there will be measurements of fluctuations of pH, oxygen concentration and salinity, partly so as to provide some basic calibrations of usefulness of chemical samples taken from the reef. The first spell in the submarine vessels will also be a chance to test different kinds of fishing gear and bait with live fish. Early on in the series of dives, there will be attempts to make maps of the region and to follow the interaction between the sea and the sea bed. Throughout the summer, however, the local lobsters will be kept under careful watch, chiefly in the hope of making behavioural studies and of understanding the relationship between the animals involved in the underwater food chain. One of the questions of particular interest is the possible relationship between chemical variables, such as oxygen or carbon dioxide concentration, and the onset of migration among animals on the sea bottom and, as a part of that study, one of the visiting teams will establish an artificial community of animals on the sea bottom.

The particular interest of the Office of Naval Research and of NASA is in the biology of the people who will at various times visit the sea bottom and for this reason there will be daily samples of air and water from those who spend time in the diving bells. The interest of blood examination is considerable and there will also be an attempt to compile from the records of the visits to the sea bottom a more accurate series of decompression tables based on nitrogen and oxygen than exists at present.


The Tektite 2 site at Great Lameshur Bay, St John Island, US Virgin Islands.

British Visitors

MR ANTHONY WEDGWOOD BENN, Minister of Technology, and Sir Solly Zuckerman, the Prime Minister's Scientific Adviser, have been in Washington this week, Mr Benn at the invitation of Mr Maurice Stans, Secretary of Commerce, and Sir Solly Zuckerman for one of his periodic informal meetings with the Office of Science and Technology at the White House. Mr Benn has been at the beginning of a tour of the United States which will take him to the California Institute of Technology, Chicago and New York. One of the questions likely to have arisen in discussions with the National Aeronautics and Space Administration is the extent to which countries such as Britain may wish to collaborate in future programmes for space exploration. Sir Solly Zuckerman's interest seems to consist chiefly of environmental problems.

Investment in Freedom

from our Soviet Correspondent

AT a time when Soviet thought centres on the forthcoming Lenin Centenary, and the achievements of Soviet science and technology are being enumerated in honour of the leader, it comes as a jarring note that so eminent a physicist as Academician A. D. Sakharov, holder of the Order of Lenin and "father of the Russian atomic bomb", should cast doubts on the validity of the Soviet technological miracle.

This is not, of course, the first time that Sakharov has expressed doubts on this topic. In his open letter of 1968 he was already sounding a warning note. Side by side with his demands for greater intellectual freedom, the abolition of censorship and the like, he drew to the attention of the planners the danger inherent in pollution both from industrial wastes and from the over-lavish use of chemicals in agriculture, and the ruining of the natural resources of the Union by "senseless despoliation caused by local, temporary, bureaucratic and egotistical interests, and sometimes simply by questions of bureaucratic prestige. . . ." Yet at the same time, he could offer a qualified comfort from the fact that, in industrial fields, the Soviet Union did seem to be slowly overtaking its rival. One should not, however (so he implied), rely on absolute figures alone, nor draw too great a comfort from the raw data. The Soviet Union was catching up with its rival chiefly in those "old traditional industries" (coal and steel, for example) which were no longer so vital a part of the US economy, whereas in newer fields such as automation, computers, petrochemicals and industrial research and development in general, Soviet progress was not only lagging but had a slower rate of growth, "so that a complete victory of our economy in the next few decades is unlikely".

One must also remember, he warned, the "ski-track" effect. When one skier starts ahead of the other, it is difficult to assess their relative strength. "The skier in the striped jacket broke the snow, while the one in the red jacket did not have to."

But in spite of these important qualifications, in 1968 he concluded that "we have demonstrated the vitality of the socialist course which has done a great deal for the people materially, culturally and socially".

And now, twenty-one months later, another such open letter is circulating from hand to hand through the academic circles of Moscow. Here the keynote is one of increasing gloom. The Soviet Union surpasses the United States in the mining of coal, true, but lags behind in oil drilling, far behind in gas drilling and in the production of electric power, hopelessly behind in industrial chemistry and is virtually a non-starter in the computer field. "One can only say that we are living in a different epoch."

If the Soviet Union is not to degenerate into a second-rate provincial power, considerable rethinking of industrial planning to meet the new age is essential. Expanding this point, Sakharov points out the very real wastage to Soviet science imposed by censorship laws. "Exchange of information with foreign countries is hampered for fear of the penetration of hostile ideology." Within the Union itself, "information on our shortcomings and unfavourable phenomena is kept secret, lest it be used for propaganda against us", while new and bold theoretical concepts and practical proposals are quashed without discussion, in case they might prove too disruptive. "The desire of the intelligentsia for greater freedom is legal and natural"—and, he implies, is also a powerful tool in economic and technological progress. The "restrictions, administrative pressure, dismissals and, even, the conducting of trials" imposed by the bureaucrats produces, therefore, an ever-widening gap between intellectuals and state-planners, a gap which, in present conditions, "must be termed suicidal".

The wastage of manpower, or rather of brain-power, due to the imprisonment, or internment in mental homes, of intellectuals, for opposition "in the field of ideas and convictions" is equally deplored. A further wastage seems inherent in the current policy towards the non-Russian nationalities within the Soviet Union. The nationality question is a large problem linked with the whole system of internal passports, without which movement from place to place is impossible, and the evidence of recent literature at least (notably the novel *The Cathedral*, by the Ukrainian novelist Oles' Honchar) suggests that the passport scheme is being used as a means of keeping promising young people "down on the farm" irrespective of whether they might perform a more productive and beneficial function elsewhere. At all events, the eventual abolition of the internal passport system is one of the fourteen points in the plan for the salvation through "democratization" which Sakharov proposes for Soviet industry and technology.

On the whole, the other points proposed are Fabian rather than spectacular in tendency. Against the suppression of distasteful, but necessary, information on the true state of the country, he counsels a "restricted distribution (via party and state organs)" of such information to those most in need of knowing. It is only later that this type of material should gradually be made accessible to everyone. The problems of "democratization" (free interchange of information, and the use even of critical minds in the planning of industry) should be, he advises, discussed through the Soviet press. The party should commit itself to the concept of democratization, which will "restore to our ideological life its dynamics and creative nature", and abolish the current "bureaucratic, dogmatic and hypocritical" attitude of officialdom.

Finally, it should be observed that Sakharov's approach seems largely pragmatic. While it is "legal and natural" to desire greater intellectual freedom, the granting of such is urged not on ethical grounds but as a practical means of providing a forward-looking and progressive technological advance into the seventies. Antidemocratic traditions, he says, are a relic of Stalinism. No longer (in another of his home-spun parables) can the lorry of progress be driven with one foot on the accelerator and the other on the brake. Current policies will lead to a technological and economic standstill. Democratization is the only sound policy. "At the present time, we still have a chance to take the right road and carry out the reforms we need. In a few years, perhaps, it may be too late !"

How to Sell the Social Sciences?

by our Special Correspondent

Paris, Wednesday

THE times are propitious for the social sciences to win funds and influence government policy. But how can the customers' good will best be exploited when on past performance the social sciences (economics excepted) have relatively little to offer to the decision making processes of government? The problem of dressing a nearly empty window was, in rather more words, the chief preoccupation of the conference held at the Unesco building in Paris this week on social science policy.

The occasion was made more than purely academic by the presence of several delegates responsible for formulating their country's research policy. Indeed, Mr Andrew Shonfield, chairman of the British Social Science Research Council, was positively optimistic about the part the social sciences have to play in government at some time in the future. Compared with economics, their present influence is on the whole trivial but then the economic indicators which have proved invaluable simply for measuring trends were only developed within the past 40 years. There is no reason why similar indicators should not also be developed in the social sciences, although, of course, the attainment of respectability is not furthered if on the rare occasions when a sociologist's views are adopted as a guide to policy his colleagues "tend to see it in terms of a sleight of hand by which one of their number has managed arbitrarily to capture and use the power of a politician, in the sort of way that Lysenko used Stalin".

An outstanding example of the philosopher becoming king is, of course, the way in which the report on negro family structure made by Mr Daniel Moynihan in 1965, when he was assistant secretary of labour, was adopted by President Johnson as the basis for a whole programme of reform. If the social sciences have so few successes of this nature to their credit, that could be because of the crudity of their theoretical apparatus when compared with the comprehensive embrace of economics. An economist who makes a recommendation on money supply, Mr Shonfield pointed out, is not surprised to be asked how this may affect the level of employment in the building industry in Northern Scotland; but the sociologist advising on a change in structure of primary schools would be unable to say how this might impinge on the operations of authority in the factories.

A different reason for the "lack of popularity of the social sciences among the decision-makers" was suggested by Dr Hemming Friif, director of the Danish National Institute of Social Research; "either the scientists are criticizing the government or they deal with matters which are looked on as esoteric to those grappling with the pressing problems facing their country". Ivory towers also loom tall in the demonology of Professor D. Goldschmidt, director of the Max-Planck Institute for Educational Research, who accused university sociologists, in Germany at least, of being more interested in establishing their subject as an academic discipline than in translating it into practice, as the student movement are rightly demanding that they should do.

If not in the method, are the reasons for this modest achievement to be sought in the men or the means? The human material was blamed in a discussion between the scientific secretary and the chairman of the Social Science Research Council at the second morning session. Mr J. Mitchell frankly confessed that although the SSRC receives only 2·6 per cent of the total budget for the five scientific research councils in Britain "we none the less have great difficulty in spending this money usefully". No less than 65 per cent of the grant applications made to the SSRC are rejected because of poor quality. Money therefore is not a restraint, but although the SSRC has spent half its budget since 1965 on postgraduate training in the hope of improving supply, Mr Mitchell avowed himself "very gloomy that we will get a substantial supply of competent research workers". His pessimism sprang in part from the belief that there is in the social sciences the lack of a clearly defined research tradition such as distinguishes the natural sciences, nor any consensus as to what is good research and what is bad research.

In response to this public washing, the work of the PEP and the Native School of Anthropology was held up as examples of British linen not being as dirty as was alleged. But Mr Shonfield agreed with his colleagues' diagnosis, at least as a general point that probably affected other countries as well. There has been quite a lot of top talent in the social sciences in Britain, he said. But the vast expansion of recent years had rushed people into professorial chairs at a premature stage in their careers. This dilution of talent was the cost of expansion.

These self-criticisms seemed to appertain to a different world to that described by Dr Henry Riecken, president of the Social Sciences Research Council in Washington, who mentioned that there are about 110,000 social scientists active in the United States enjoying some 800 million dollars of support from federal funds, foundations and other sources.

What use have the social scientists made of this kind of money? Professor A. B. Cherns of Loughborough University, the organizer of the conference, pointed out that federal expenditure on research in the social sciences amounted to an estimated $333 million in 1968, but that, in response to congressional enquiry, agencies were unable to describe the use made of previous research. But stewardship in the social sciences, Professor Cherns argued, is not just a matter of talent. "Research can only have a specific use if it is conceived as taking a narrow view of the problems it is tackling."

On the showing of the conference it may be hard to define what assistance the policy-maker can legitimately expect from the social sciences, or on what lines social science policy should be planned. But then, as Professor B. Suchodolski of Warsaw remarked, "We have never had any example of state government by sociologists and it might be doubted if such a vista for the future would be a tempting one".

NEWS AND VIEWS

Basic Questions about Moon still in Doubt

Nobody really expected the lunar samples to solve all the problems of the solar system in one swoop, yet it is surprising how little basic understanding of the Moon has been revealed since Apollo 11 nine months ago. This was clear at the NATO Advanced Study Institute on the Moon and Planets which began at the University of Newcastle upon Tyne last week.

It is true that a vast amount of raw information is available in the form of 120 pounds of rock and soil returned so far. There is also the information produced by the instruments left behind on the surface of the Moon, although nobody knows quite what to make of the seismometer results. And on top of the information obtained at first hand there are the data from a decade of unmanned probes to the Moon, culminating in the Surveyor and Orbiter series in the late 1960s.

But basic problems such as the nature of the interior of the Moon and the forces which moulded the lunar surface are still open to vigorous discussion, as the Newcastle conference is demonstrating. And, as well as the failure to answer the questions about the Moon and the solar system in general which have always been a puzzle, the space programme has set further problems which the samples have so far failed to answer. Nobody would have it otherwise, of course.

The concentrations of mass under some of the ringed maria, which close analysis of the paths of spacecraft in orbit around the Moon has revealed, are one example. Another is the nature of the soil which blankets the parts of the Moon visited so far to a depth of metres. It seems clear from the composition of the soil that it could not have been created by the grinding up of the igneous rocks which have been found on the Moon, including the anorthosite which is suspected to be the chief component of the highlands. The age measurements—$4\cdot65\times10^9$ years for the soil compared with a range of $3\cdot59$ to $3\cdot93\times10^9$ years for the rock samples—also argue for the uniqueness of the soil and are a further obstacle to a simple explanation.

Naturally enough, the discussion centres on the nature of the interior of the Moon, and here the mass concentrations are taken to be the primary clue, although the weak magnetization of the samples is an important indicator seeming to imply that the Moon once had a conducting liquid core.

Much depends on the extent to which the gravity maps produced from the Orbiter data are to be trusted, but it seems that the contours are more like what would be expected if the mass is concentrated in plates under the maria than the prediction for spherical bodies buried under the surface such as slow-moving meteorites might conceivably produce. This is a question which requires the tracking of spacecraft in low altitude orbits which are more susceptible to gravitational inhomogeneities than the 100 km orbits of the Lunar Orbiter series. This is something for later Apollo missions. If the mass is indeed concentrated into plate-shaped structures, then this is a strong argument in favour of the view that the gravity effects can somehow be explained in terms of the evolution of pools of lava. Yet some people believe there is a problem in accounting for the way in which the mass is supported, and suggest that this implies a cold lunar interior. The evidence for molten material at the surface must then be explained by a process such as heating by the Sun during an early superluminous phase.

The question which is beginning to surface in people's minds is whether the exploration programme which has been laid out to 1974 is going to supply the information to solve the problems which are still outstanding. It may be that material from the diverse sites which have been earmarked for exploration will throw a new light on the data which have so far been accumulated purely from landings in the maria. There is the possibility of picking up material from deep within the Moon which has been thrown out by meteorite impact, for example. This must be what people are hoping, if the fundamental questions about the nature of the Moon are to be answered.

Mapping the RNA Phage

Molecular biologists, always in pursuit of new clues to the mechanisms of protein synthesis, are at present very excited about RNA bacteriophages. This enthusiasm springs from the remarkable ease with which the *in vivo* situation can be mimicked in the test tube when these viruses are used. A flood of recent articles has been concerned with the nucleotide sequences of the genes themselves, the structure and function of the punctuation signals between the genes and the ways in which the translation of one gene may control the translation of others by causing changes in the secondary and tertiary structure of the RNA. On page 230 of this issue of *Nature*, Jeppesen, Steitz, Gesteland and Spahr report the first unambiguous determination of the order of the genes of phage R17.

RNA phages code for three proteins, phage coat protein, maturation (A) protein and RNA synthetase enzyme. Because there is no genetic recombination between RNA phages, it is not possible to map their gene order by conventional genetic analysis. Attempts have been made, therefore, to achieve mapping by following the way in which they direct the syntheses of their three protein products. This approach has depended on the assumption that their translation is similar to that of messenger RNA of bacterial operons, and in hindsight it is now clear that this assumption is unjustified and has led ineluctably to the conclusion of incorrect gene order.

When a bacterium is infected by phage, different amounts of the three phage proteins are produced, roughly in the proportions 20 : 5 : 1 coat : synthetase : maturation. This led to the suggestion that the order of genes from the 5′ to the 3′ end of the molecule in the phage was coat–synthetase–maturation, ribosomes attaching only at the 5′ end and sequentially translating the messenger, but with some ribosomes dissociating from the messenger at the end of each gene so that decreasing amounts of each protein are produced. This is consistent with the finding that the translation of the coat protein gene is necessary before it is possible to translate the synthetase gene. This theory had to be modified when it was found that, although synthetase protein is not produced until some time after infection, both coat protein and maturation proteins are produced almost immediately (albeit in different quantities). Thus it was suggested that ribosomes can attach to both the coat gene and the maturation gene (although with a lower affinity in the latter case), but cannot do so to the synthetase gene; this is translated only by ribosomes which have passed into it after translating the coat gene.

Unfortunately for this model, recent work has shown that each of the three phage genes commences with an initiation sequence of nucleotides which can bind ribosomes directly. If a complex of ribosomes–phage RNA is digested with nucleases, these initiation regions are shielded against the enzymatic degradation and remain intact. They can then be isolated and characterized by the oligonucleotide digestion fingerprints produced by treatment with T1 ribonuclease. Each of these three initiation regions has been identified in this way (*Nature*, **224**, 957; 1969).

Jeppesen *et al.* have made use of this finding to produce a genetic map of phage R17 by biochemical analysis. The enzyme ribonuclease IV from *E. coli* cleaves the phage RNA at a position about 40 per cent along the molecule from the 5′ end. The initiation regions present on each of the fragments were identified by degrading the fragment–ribosome complexes with nucleases and characterizing the protected regions of each. The maturation protein gene initiation sequence is present in the 40 per cent fragment only; the coat protein gene initiation region also appears primarily in this fragment, and the synthetase gene initiation region is present primarily in the 60 per cent fragment.

These data make possible unambiguous determination of the biochemical map of the phage. The coat protein gene must lie in the middle of the messenger because the oligonucleotide T1 fingerprints which correspond to the 5′ end of its initiation region appear in the 40 per cent fragment, whereas nucleotide fingerprints corresponding to internal parts of this gene appear in the 60 per cent fragment. The precise site of attack of ribonuclease IV seems to lie in the nucleotide sequence which codes for the first six amino-acids of the coat protein. The highly localized yields of the initiator sequences of the other two genes show that the maturation must lie in the 40 per cent fragment and the RNA synthetase in the 60 per cent fragment. In agreement with this conclusion is the previous finding (*Proc. US Nat. Acad. Sci.*, **59**, 879; 1968) that when the two fragments are used to direct protein synthesis *in vitro*, the 40 per cent is relatively inactive as messenger, but the 60 per cent efficiently directs the synthesis of synthetase protein. Coat protein is not produced efficiently by either fragment. Thus the order of genes in the phage must be: 5′-maturation–coat–synthetase-3′.

This comprises the final nail in the coffin of the coat–synthetase–maturation gene order model. How then is the differential expression of the three phage genes to be accounted for? It seems that ribosomes can bind directly to the initiation regions of each gene; perhaps this binding may be of differing efficiency in each case. This is supported by the different yields which are obtained of the three initiation regions. The affinity of the regions for ribosomes is probably influenced not only by their particular nucleotide sequences, but also by the secondary and tertiary structure of the phage RNA. That the coat protein gene must be translated before the synthetase gene can be translated can now be interpreted to imply that changes in the secondary and tertiary structures may be brought about by the binding of ribosomes to the coat gene initiation region; these changes could control the extent of availability of the synthetase initiation region.

MICROSCOPY

Thinking Small

from our Molecular Biology Correspondent

EVEN those physical chemists or biochemists to whom the very idea of trying to see a molecule, or even its incinerated shell, savours of cheating have had to recognize that the electron microscope is here to stay. That the modern instrument is fast approaching the theoretical limits of resolution has been some reassurance, but recently a sinister new threat to their livelihood has been taking shape. This is the scanning microscope which Crewe in Chicago had been developing. An account of the principles of the design in comprehensible form for the general reader, and some examples of what the machine can do, are now available for public contemplation (Crewe and Wall, *J. Mol. Biol.*, **48**, 375; 1970).

In the scanning microscope, as commercially available, the specimen is traversed by a focused electron beam, and it is the secondary electrons emitted by the sample that are detected. It is a blunt instrument, however, limited by the emissivity of the tungsten filament source, which produces a spot of not less than about 100 Å. Crewe and Wall nevertheless opted for the scanning principle as a basis for a high-resolution instrument, partly because the focusing system makes it possible to operate on a very small field, and also because of the important feature that the contrast need not be linked to resolution. They work, however, with the electrons transmitted by the sample, rather than the secondary emission. A central technical problem was to devise a source capable of producing very high intensity in a small spot. The electron gun design was based on the metal tip field emission source, which gives about a million times the brightness of a conventional hot filament, and can be focused to give a spot of about 5 Å. Many other formidable technical problems and their conquest are described by Crewe and Wall, though from their description it seems that at least in a manipulative sense their instrument is simpler to align and use than a conventional electron microscope.

The results show great promise. A picture of a T4 bacteriophage shows a high degree of detail in the tail sheath; in rods of tobacco mosaic virus, stacked protein disks are resolved with great clarity; and in single molecules of the protein ferritin, the subunits and the ferric cores can be clearly discerned.

A further facility that the instrument offers is measurement of the energies of the transmitted electrons. The energy loss on passing through a specimen is a function of the material, and the measurement of energy loss distribution should thus open up analytical applications, as well as the possibility of increasing the contrast by choosing the most favourable energy band for detection.

Electron microscopists will no doubt be waiting anxiously to see whether the promise of high contrast at the best resolution is fulfilled; in a new review also just published (*Quart. Rev. Biophys.*, **3**, 137; 1970), Crewe has already reported the probable detection of single chemical substituents, containing clusters of heavy atoms, in DNA, so that the strategy evolved by Beer and his colleagues of sequencing nucleic acids with the electron microscope may soon be realized.

ENDOCRINOLOGY

Hormones in Cells

from a Correspondent

An eminent collection of people with an interest in hormones met in Bristol from April 5 to 11 to discuss subcellular organization and function in endocrine tissues. Professor Marilyn Farquhar (University of California School of Medicine, San Francisco) described how in the adenohypophysis excess hormone-containing granules are incorporated into lysosomes. Future research may well centre around control systems determining intracellular recirculation of granules to the Golgi apparatus and the direction of granule transport in cells.

Professor I. Geschwind (University of California, Davis) indicated that a possible site of action of calcium during its mediation of the secretory process was on the intracellular microtubular system evolved to direct granules to specific sites on plasma membranes for secretion. Later, Dr E. K. Matthews (University of Cambridge) suggested that calcium induced secretion by reducing the intracellular viscosity and the energy barrier arising from the surface charge on the granule and the inner face of the plasma membrane, thereby permitting fusion of the granule and cell membranes.

Professor W. W. Douglas (Yale University Medical School, New Haven) provoked much discussion with morphological evidence which apparently indicated that the neurohypophyseal hormones are secreted by exocytosis. This would involve the inward budding of the plasma membrane, subsequently transported centripetally as microvesicles. There were doubts as to whether this rarely observed process of a two-way axonal or cellular transport system of hormone storage and microvesicles could be the physiologically significant route for granule secretion. The alternative between the existence of an "extragranular hormone pool" and exocytosis as mechanisms for hormone release was discussed by Dr R. P. Gould (Middlesex Hospital Medical School, London), who suggested that the site of fusion of the granule and cell membrane could become an area of increased permeability permitting the outward movement of high molecular substances.

Drs F. S. LaBella, S. Shin and S. R. Vivian (University of Manitoba, Winnipeg) reported the existence of six neurophysins, but the consensus of opinion on present evidence settled the number at three. During a session on neuroendocrine organization detailed comparisons were made between neurosecretory systems in invertebrates and vertebrates. It seems that comparative research determining possible carrier proteins for neurohypophyseal hormones in vertebrates may prove rewarding and of significance in understanding the vertebrate system.

Professor Dorothy Hodgkin (University of Oxford) described the most recent model of the structure of insulin. She also commented on correlations between species amino-acid differences in the insulin molecule which are located on the "exposed" surface of the molecule with differences in the hexagonal laminated structure of the insulin granule. She agreed with Professor H. Heller (University of Bristol) that the molecular configuration of protein and peptide hormones and their carriers may well have a direct effect on the morphology of the hormone granules, thus providing a "bridge" between molecular and ultrastructure.

In a session devoted to catecholamines Dr J. Häggendal (University of Göteborg, Sweden) presented some new information about the uptake of exogenous noradrenaline chiefly by newly formed amine storage granules. Dr A. Pletscher (Hoffmann-LaRoche Co., Basle) had evidence for the storage of biogenic amines *in vivo* as high molecular weight aggregates of ATP, produced by stacking together molecules of the nucleotide. Professor J. P. Green (Mount Sinai School of Medicine, New York) considered the reactive sites on the histamine (the amino-acid group of the side chain and pyridine-like ring nitrogen) and 5-hydroxytryptamine (primary amino group of the side chain and specific electrons of the indole ring) which would form linkages for binding in granules.

NUCLEIC ACIDS

Precursors and Products

from our Cell Biology Correspondent

Messenger RNA molecules undoubtedly exist in the cytoplasm of eukaryotic cells, but precisely how they get there is something of a mystery. In the early nineteen sixties it was tempting to assume that the heterogeneous and high molecular weight RNA which occurred in nuclei and was rapidly labelled with short pulses of labelled bases was mRNA. After all, heterogeneity of size and rapid turnover are characteristics expected of a pool of mRNAs. But between 1963 and 1966 it was proved to everyone's surprise that most of this rapidly labelled nuclear RNA never leaves the nucleus let alone functions as messenger programming cytoplasmic polysomes.

The picture has changed little since then and the latest attempt to prove a relationship between heterogeneous nuclear RNA and mRNA (Soreiro and Darnell, *J. Cell Biol.*, **44**, 467, 476; 1970) is, like its predecessors, ambiguous. To decipher the events between synthesis of mRNA and its functioning in the cytoplasm it is necessary to follow the fate of a specific mRNA, and so far this has proved impossible. At present the best that can be done is to seek common base sequences in mRNA fractions and any putative nuclear precursor, such as heterogeneous nuclear RNA, by competitive hybridization experiments. The great snag to this approach is that molecules with related but not identical sequences competitively hybridize with DNA as if they had identical sequences. Even after taking all precautions to maximize the resolution of this technique, as Soreiro and Darnell have done, the most that can be said is that mRNA and heterogeneous RNA have similar base sequences. Similar amounts of the two RNAs from HeLa cells bind to a given amount of DNA and presaturation of the target DNA with cold heterogeneous RNA reduces the amount of hot mRNA or heterogeneous RNA that can hybridize. A small fraction of the heterogeneous RNA may therefore be a precursor of mRNA if the heterogeneous RNA molecules are cleaved before migrating to the cytoplasm to act as messenger.

The intranuclear maturation of ribosomal RNA is the obvious precedent for a maturation process involving specific cleavage. According to Weinberg and Penman (*J. Mol. Biol.*, **47**, 169; 1970), in HeLa cells infected with polio virus (when infection induces the accumulation of ribosomal precursor RNAs) the chief route of maturation includes four steps. A 45S precursor containing the sequences of both 18S and 28S ribosomal RNA is synthesized only to be cleaved to yield a 41S molecule and a small fragment which is degraded. The 41S molecule is cleaved again into a 20S and 32S molecule; these finally give rise to 18S and 28S ribosomal RNAs when short fragments are clipped off. The whole process boils down to elimination by specific cleavages of nearly half the original 45S precursor.

The next step in ribosome assembly, the association of ribosomal proteins to ribosomal RNA, also proceeds in a stepwise fashion, giving rise to discrete subribosomal particles. In *E. coli* the ribosomal proteins fall into at least three groups, each associating at a characteristic rate. Various values for the half-lives and pool sizes of individual ribosomal proteins have been reported and there is a modicum of agreement with the differences perhaps attributable to differences in culture conditions and strains used. Sells and Davis (*J. Mol. Biol.*, **47**, 155; 1970), for example, recently reported half-lives of individual ribosomal proteins ranging from 2 to 11 min with pool sizes varying from 1·7 to 9·4 per cent of the total ribosomal protein pool.

A particle sedimenting at 43S seems to be the immediate precursor of the 50S ribosomal subunit. Forget and Varrichio (*J. Mol. Biol.*, **48**, 409; 1970) believe that in *E. coli* K12 growing at its maximum rate the availability of protein rather than some metabolism of the ribosomal RNA limits the rate at which 43S particles become 50S subunits. They found that the 43S particle has the same 5S/23S RNA content as the mature subunits although the 5S RNA is less firmly bound to the precursor. Moreover, they could not detect any differences in the chemistry of the RNAs from precursor particles and mature subunits. At least in conditions of maximum growth the supply of proteins therefore seems to be the bottleneck.

PHYTOCHEMISTRY

Terpenoids for Progress

from a Correspondent

KNOWLEDGE of the terpenoids is growing fast, as members of the Phytochemical Society showed when they met in Liverpool on April 7 to 10 to discuss the biochemistry of these compounds. Many speakers pinpointed an important recent development when they demonstrated convincingly that polyisoprenoids in their many forms can no longer be ignored as "end products" or backwaters of secondary metabolism. The importance of the terpenoids in ecological interactions was highlighted by Professor R. B. Clayton (Stanford University), who said that such informationally rich compounds may well have contributed to evolution from prebiotic times. Another notable theme of the meeting was the analogy between reactions in terpenoid biosynthesis and well known transformations in organic chemistry, for example, in the cyclization of squalene 2,3-epoxide to lanosterol.

Dr A. Pfiffner (Hoffmann-LaRoche Co., Basle) described a series of syntheses of the eight geometrical isomers (all racemates) of the juvenile hormone of insects. Biological assays carried out with each isomer showed that usually the natural isomer was the most active, but there were species differences. The 2-*trans*,6-*cis*,10-*cis* isomer turned out to be more effective in causing sterility in some insects.

Dr M. J. O. Francis (University of Oxford) suggested that monoterpene glycosides, which are equally soluble in organic solvents and water, would provide a plant with an ideal mechanism for glucose transport. This would be analogous to the function of polyprenol phosphate in bacterial cell wall synthesis.

Dr J. Macmillan (University of Bristol) underlined the importance of the work of West *et al.* in solving major enzymological problems in gibberellin biosynthesis, with the result that, although much remains to be done, the overall pathway (at least in fungi) seems fairly clear. One reaction he mentioned which has no counterpart in organic chemistry is the hydroxylation of a tertiary (bridgehead) carbon atom.

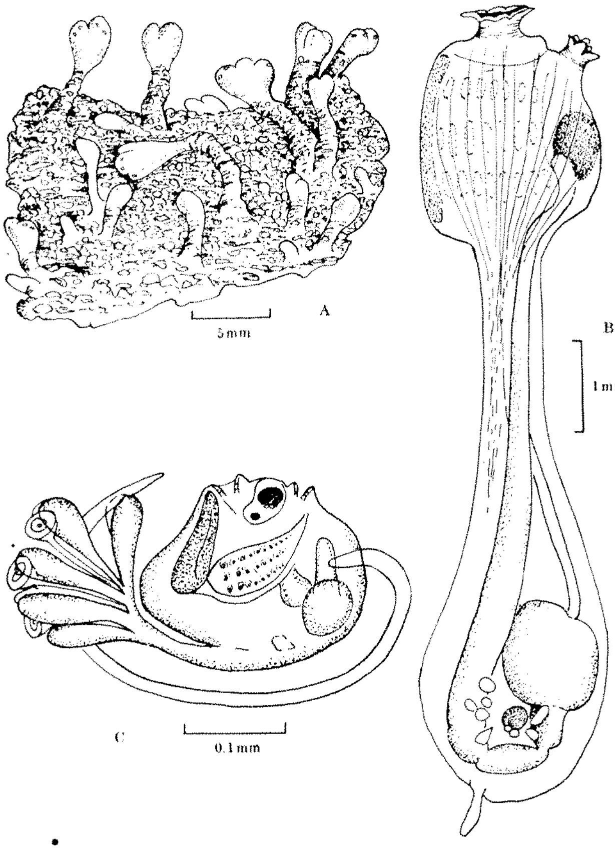
Professor T. W. Goodwin discussed the thesis that only by comparing carotenoids produced at the end of the proposed biosynthetic pathways (rather than the intermediates) could significant conclusions be drawn when interrelating the species that contain them (in this case the algae). Dr S. L. Jensen (Institute of Technology, Trondheim) insisted that many recently discovered carotenoid structures which have been in part deduced from mass spectral data must be confirmed by synthesis. Several speakers also made it clear that pathways deduced from the patterns of naturally occurring compounds of related structure can only be regarded as tentative when evidence is lacking at the enzyme level.

The notable recent increase in new structures was illustrated by Professor V. Herout (Academy of Sciences, Prague), who dealt with the sesquiterpenes: more than 100 types of carbon skeleton are known in this group, which must present a rich supply of experiments for biosynthetic work.

MARINE BIOLOGY

Underwater Guide

An eagle eyed underwater zoologist who recognizes these creatures attached to a nearby rock should know that he is in the vicinity of Plymouth or North Carolina, or perhaps off the coast of France. This detail of geographical distribution he would have learnt from R. H. Millar's new handbook, *British Ascidians*, the latest of the Linnean Society's synopses of the British fauna (Academic Press, £1 2*s*). The book contains keys to the families and species of ascidians to be found in British waters, as well as notes about individual species. This illustration, one of many, shows (*A*) part of a colony, (*B*) a zooid and (*C*) a larva of *Archidistoma aggregatum*. (Several zooids are housed within each projection on the colony.)



THERMODYNAMICS

Keeping Up to Date

from a Correspondent

Thermodynamics still holds surprises, as delegates learnt at the international conference on this topic held at Cardiff on April 1–4, under the auspices of the Institute of Physics and the Physical Society. The organizers judged correctly that the survival value of the subject lies first in its breadth (delegates included chemists, engineers, mathematicians and physicists) and second in its adaptability. Thermodynamicists are willing to dilute the classical purity of their doctrines and open judicious doors to new recruits. For example, it is possible that they are making a take-over bid for parts of cosmology. Dr D. Layzer (Harvard) presented a fascinating account of how the cosmological "arrow of time" could be linked with thermodynamic irreversibility by arguing that initial conditions in a Friedman universe were probably such as to generate high initial information and low entropy. The subsequent increase in entropy is then linked to the growing information embodied in history. Layzer's viewpoint is epitomized in his remark: "Structure and order are tricky. We should keep to well-defined concepts like information."

Well within the classical tradition was a new sharpening of Gibbsian thermodynamics which dealt in some detail with phase changes and solution processes occurring in elastically stressed systems. This extension, by Dr A. G. McLellan (Christchurch), has been made by defining suitable extensive coordinates which describe the shape of a crystal. Successful applications have been made to the α–β quartz transition, crystal twinning and homogeneous diffusion. The work is likely to have important applications in geophysics.

The other classical topic discussed at some length was the question of axiomatics. Dr R. Giles (Kingston), who several years ago provided the first really fresh approach to thermodynamics since the time of Carathéodory, was urging a pedagogical version of his system on a somewhat unwilling audience, most of whom sympathized with the remark of Dr M. W. Zemansky (New York): "Work is anathema. In Britain, I sometimes think that heat is anathema. But work and heat are here to stay." Dr W. J. Hornix (Nijmegen) reported progress with the (axiomatically) least satisfactory aspect of thermodynamics—the treatment of chemical systems of varying composition.

Much was said about irreversible thermodynamics which has been part of the extended domain for at least twenty years. The two chief branches of this subject are the mathematical neo-Cauchy theories expounded, for example, by Truesdell, and the physico-chemical theories of relaxation and transport started by Onsager. No significant new contribution was offered at Cardiff, but it seems clear that both subjects are still moving, presumably because people have faith that their unfulfilled promise will be redeemed.

There were interesting accounts of dynamical calculations on finite models which illuminate the passage to equilibrium in isolated systems. Typical of these was work by Dr J. Ford (Atlanta) in which two or three oscillators were coupled by non-linear resonant interactions. The Arnold stability of orbits can be dramatically broken in a way which renders the motion substantially ergodic. This has been shown by machine computation of the orbits. At the other extreme Dr S. Fujita (Buffalo) announced a new formulation of the many-body problem in which the infinite hierarchy of coupled equations is replaced by a single closed equation for the one-particle distribution function. As he remarked: "There are many, many methods, and you can use any one of them to derive desirable results."

There were some sad absentees: nobody said anything substantial about biological thermodynamics, nor about critical phenomena, which received a brief but illuminating mention in a survey by Dr L. Tisza (Cambridge, Massachusetts). But the full proceedings, which are expected to be published in July, should include an important paper on the analytical description of critical points by Dr M. H. Coopersmith (Virginia), who was not able to read his paper at Cardiff.

JOSEPHSON EFFECT

Making Way for a New Volt

by our Solid State Physics Correspondent

THE Weston cell may be on its last legs as an international standard of voltage because of the rapid progress in developing high precision measurements of the a.c. Josephson effect. T. F. Finnegan, A. Denenstein and D. N. Langenberg (*Phys. Rev. Lett.*, **24**, 738; 1970) have wrought several changes in the apparatus which provided W. H. Parker *et al.* (*Phys. Rev.*, **177**, 639; 1969) with a value for the ratio *e*/*h* to a precision of 2·5 parts in a million, and have improved on the accuracy by a factor of five. This puts the measurements within touching distance of the accuracy claimed for voltage determinations from Weston cells and makes it virtually certain that the cell will soon be ousted as a voltage standard in favour of the far more elegant solid state device.

The real attraction of the a.c. Josephson effect, in which the voltage across a junction varies with the frequency of the supercurrent, is its independence of the shape of the junction. Different sets of apparatus will give the same characteristics, as Parker *et al.* found in their determinations of the ratio *e*/*h*, which relates the frequency to the voltage. Frequencies, moreover, are very easy to measure accurately, being independent of temperature and extraneous effects. What Finnegan *et al.* have achieved is to increase the precision of the experiment to the point at which the ratio can be redefined as an auxiliary constant, allowing a voltage standard to be established in terms of frequency.

Comparison of the junction voltage with a reference cell provides the limiting accuracy in *e*/*h*. In previous determinations a conventional potentiometer was used with adjustable resistances, but Finnegan *et al.* have capitalized on the potential of a Josephson junction as a variable voltage source by allowing microwave radiation of a chosen frequency to interact with the supercurrent to give the desired voltage. A fixed ratio resistance network can then be used. They have also managed to raise the Josephson voltage by a factor of ten, to 10 mV, which reduces noise factors and lead resistance corrections and improves the voltage ratio with the standard cell. With further modifications, they are confident that *e*/*h* will soon be known to less than 0·1 p.p.m. and see attention shifting to the problem of how to disseminate the standard volt, once established in a Josephson device, to different parts of the globe.

The results of Finnegan *et al.* are likely to be discussed at the forthcoming conference at Gaithersburg, near Washington. Similar experiments at the National Physical Laboratory are expected to be ready in time for the conference on atomic masses and related constants at the NPL in September 1971, but it seems that the fate of the volt will have to await the next international meeting on standards due to be held at Sèvres, near Paris, some time within the next five years. Certainly the present system in which the ampere, measured by the force between two wires, is taken as the primary electrical unit will come under fire. The ampere can only be reproduced to an accuracy of about 4 parts in a million.

The fine structure constant α is related to *e*/*h* by the gyromagnetic ratio of the proton. Finnegan *et al.* point out that if α could be measured to better than one part in a million, their new value for *e*/*h* would provide a superior determination of the gyromagnetic ratio than that available from direct experiments.

PHOTOGRAPHY

Shock Tactics

from a Correspondent

A COMMON interest in shock wave phenomena brought together the Association for High Speed Photography and the Shock Tube Liaison Group at Liverpool between April 6 and 8. Early in the meeting a cautionary note was struck by Professor P. L. Clemens (von Karman Institute for Fluid Dynamics, Brussels) when he discussed the analysis of schlieren photographs. The marked lack of correlation in data obtained from these photographs of the flow about very high speed bodies, particularly in the wake region, indicated a requirement for a general sensitivity definition, he said. Clemens formulated such a definition, taking account of the human element in the form of the sensory nature of vision, and including the behaviour of the photographic materials and the optical arrangement of the schlieren system.

Holography attracted considerable interest, and applications to the study of shock phenomena and to the investigation of small particles moving at very high speed were described. Dr J. C. W. Gates (National Physical Laboratory) discussed the use of pulsed lasers and holographic recording in shock tubes and wind tunnels. The potential advantages included the study of variations in three dimensions from a single record, and also making several more or less independent measurements from one record by multiple reconstruction. From the economic point of view it may not be necessary to buy expensive optical equipment for this type of work because some techniques are not very sensitive to the quality of the optical components.

Another form of diagnostic technique used in shock tubes is to photograph the movement of very small particles which are convected in the flow. In the method developed by Professor J. M. Dewey (University of Victoria, British Columbia, Canada), cigarette smoke is induced to form thin trails across a shock tube, and the variation of all the physical parameters behind an unsteady shock can be determined from an analysis of the trajectories of the smoke particles within the flow. This method compares favourably with others and seems to be slightly better than piezo-electric pressure transducers, for example.

Although the new visualization techniques add further dimensions to flow analysis, the more conventional methods continue to produce important data. This was clearly illustrated by the study of the highly complex structure of the detonation wave described by Dr D. H. Edwards (University of Aberystwyth). From an analysis of schlieren, interferometer and soot-track records he has deduced that although the Chapman–Jouguet model may be adequate for predicting the gross properties of the processes, the extremely intricate wave interactions that occur behind the wave make it impossible to relate detailed structure from one set of conditions to another.

Dr J. H. L. Ranson (Pilkington Bros. Ltd) described the effects of shock propagation through windscreens toughened in certain areas leaving specially selected zones of less tough glass. In practice these shocks might be produced by stones, but in the laboratory small detonators fixed to the windscreen produced the required impact effect. Using a high-speed camera running at 200,000 frames per second it was possible to investigate the reflexion and deflexion of the shocks by the various zones. The intention is to produce windscreens through which there will still be a measure of visibility even after an accident such as that simulated by the detonator.

NEURAL DEVELOPMENT

Visual Stimuli specify Connexions

from our Experimental Psychology Correspondent

ENVIRONMENTAL events can influence the formation of neuronal connexions during development. This is the conclusion of R. M. Gaze, M. J. Keating, G. Székely and L. Beazley (*Proc. Roy. Soc.*, B, **175**, 107; 1970). The connexions made by growing neurones during development and regeneration have long been thought to be determined genetically. It now seems that this genetic determinism, which specifies where neurones make connexions in the visual system of the *Xenopus* toad, is accompanied by a means for bringing neurones together if they receive a similar pattern of input from both eyes simultaneously.

This hypothesis, due to Keating (*J. Physiol.*, **198**, 75*P*; 1968), has been tested by operating on the eyes of embryos before the formation of the retina, or any connexion of the eye with the brain. At this stage, however, the cells destined to develop into the retina were already specified as to their relative positions. In the first type of operation, a developing eye cup was cut in half vertically. The outer (temporal) half was removed and replaced by a nasal half from the opposite eye of another animal. The resulting preparation had one normal and one double-nasal eye. In the second type of operation, one of the larval eyes was rotated by 90 or 180 degrees.

After metamorphosis of the operated animals the projections of the retina onto the brain were mapped, by finding the position of a light in the visual field from which a response could be recorded electrophysiologically in each of a series of standard positions covering the optic tectum (a primary visual region of the brain in amphibia). In the normal visual system of these species there is a direct projection of each eye to the tectum on the other side of the brain (contralateral). There is also a projection from each eye to the tectum on the same side (ipsilateral), but this connexion is made indirectly through the contralateral projection to the other side. A given tectal position thus corresponds to a single point in visual space as seen by both eyes, and these points on the tectal surface provide an orderly map preserving the spatial relationships of the visual field.

Gaze *et al.* confirmed that in animals with one double-nasal eye, the primary (contralateral) projection is systematically altered. Each part of the eye in the new temporal position projected not in the way that a normal temporal retina would but in a manner appropriate to its origin on the nasal side of the eye. In contrast, every part of the indirect ipsilateral projection of the double-nasal eye was appropriate not to its embryological place of origin but to its new position with respect to the visual field.

Results from animals with one rotated eye followed the same kind of pattern. The map of the rotated eye onto the contralateral projection of the tectum was rotated appropriately to the early embryological coordinates of the retina, but inappropriately to the new retinal orientation with respect to the visual field. The ipsilateral projection, however, was not rotated, and was appropriate to the visual field.

In both types of preparation, although the contralateral projection of the normal eye was normal, as expected, the ipsilateral projection was rearranged to fit in with the altered projection of the surgically manipulated eye on the other side. Although the direct contralateral projections of both normal and abnormal eyes seemed to be determined by innate specification according to their original position in the embryo, as far as the indirect ipsilateral connexions were concerned, neurones growing from one tectum to the other appeared to have joined with those directly projecting neurones from the other eye which subserved the same point in visual space. There were thus two ipsilateral connexions in each toad, one from the operated eye with an abnormal first (contralateral) stage and a normal cross-tectal second stage, and another with a normal contralateral stage and abnormal destinations for the fibres of the cross-tectal stage.

Gaze *et al.* claim that "the evidence appears to establish beyond doubt that a functional interaction between the visual inputs from the two eyes, occurring at the tectal level, operates in the establishment of specific neuronal connexions in the ipsilateral visual projection". Any hypothesis of purely genetic determination would predict that neurones subserving the two eyes would come together according to their original embryological position (as happens in the contralateral projection). Instead, for the indirect ipsilateral projection the determining factor is a position in the visual field present during the seeing life of the individual animal. This conclusion seems to indicate that permanent connexions between two neurones can be made on the basis of simultaneous coincidence of input in both. As a principle for understanding development, and as a clue to the possible nature of the neuronal changes underlying learning, this conclusion is of the greatest importance.

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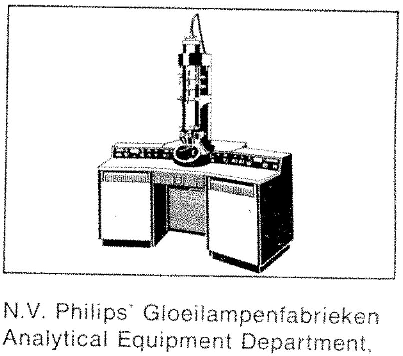

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Interrelations of Some Pituitary Hormones

by

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A comparison of the structure of melanocyte stimulating hormone (MSH) of the dogfish with mammalian MSH, adrenocorticotrophin and lipotrophin points to some possible interrelationships and lines of evolution.

At least ten hormones with different functions have been prepared from extracts of mammalian pituitary glands, and they can be divided into groups on the basis of their cytological origin, target organs and tissues and structure and chemistry. Little is known, however, about these aspects of the pituitary hormones of non-mammalian vertebrates[1]. Even the homologies of the different regions of the pituitary gland in different classes of vertebrates are sometimes in doubt and there have been few reports of the structure of the hormones, with the exception of the octapeptides of the neurohypophysis. The chemistry, pharmacology and physiology of the neurohypophysial hormones have been investigated in both mammalian and non-mammalian vertebrates, and this work has been the subject of recent reviews[2-4] in which the relationships between the peptides in different classes of vertebrate have been considered.

The adenohypophysial hormones adrenocorticotrophin and melanocyte stimulating hormone (MSH) have also been widely studied, but their chemical compositions and structures have been determined only for a few species of mammals[5,6]. Lipotrophin, the pituitary hormone responsible for the mobilization of lipids, has been fully described recently in the sheep, ox and pig. It is clearly a member of the same group of polypeptides as the other two hormones[7-9]. The biological property which this group of hormones has in common—the ability to cause the dispersion of melanin granules in the melanophores of certain lower vertebrates—was first described in pituitary gland extracts by Hogben and Winton[10]. Early work was concerned with colour change in amphibians, but it was not long before the same principle was demonstrated in the pituitaries of other vertebrates including mammals. Studies of the melanocyte stimulating hormones of all classes of vertebrates still depend on visible changes in the melanophores of a few species of reptiles and amphibians, for these are the only biological tests available. No function has yet been unequivocally ascribed to the MSH in mammalian pituitary glands[11,12]. In the dogfish, as in amphibians, the skin becomes pallid after the removal of the intermediate lobe of the pituitary and can be darkened again by injecting homologous or heterologous pituitary preparations containing MSH (ref. 13). In bony fishes and reptiles the response is complicated by direct innervation of the melanocytes. There has been some dispute as to whether direct nervous control or a melanin aggregating hormone is involved in the melanocyte response in elasmobranchs, and the evidence was reviewed by Waring[14]. The slow swimming action of the dogfish and its poorly developed escape reaction suggest that an ability to merge with the background must have survival value.

MSH in Dogfish

In spite of much knowledge of the chemistry of the mammalian MSHs, virtually nothing is known about the structure of MSH from lower vertebrates. Such information could throw light on the origin and evolution of the hormone or on its function in the higher vertebrates and so we decided to investigate MSH in an elasmobranch of commercial importance and therefore ready availability—the dogfish *Squalus acanthias*.

We first extracted MSH. The neuro-intermediate lobes from 4,000 pituitary glands were homogenized in ice-cold 1 M acetic acid. Saturation with ammonium sulphate precipitated inactive material and the supernatant was desalted in acetylated dialysis tubing[15] against 1 M acetic acid at 4° C. The freeze-dried extract was subjected to gel filtration at 4° C on 'Sephadex *G*-25'. Monitoring the eluate at 280 nm and testing each fraction for MSH activity by a modification of the method of Burgers[16] revealed a single main peak of extinction coincident with the biological activity. This peak was chromatographed on 'Biogel *P*6' and the aromatic retarding characteristics of this gel brought about further purification. Ion exchange chromatography on carboxymethylcellulose (CMC) separated the active material into two components of different basicity. These were present in similar amounts and had the same specific activity in the assay; they were termed PI and PII. The amino-acid sequences of the two components were determined by standard methods of end group analysis: subtractive Edman procedure, Edman-dansyl procedure[17,18], and digestion with amino-peptidase M and with carboxypeptidases A and B. The peptides were fragmented by digestion with trypsin and by the cyanogen bromide reaction[19], and the products were separated for further analysis using paper electrophoresis[20] and ion exchange chromatography on CMC. The sequences of the two active peptides were found to be identical and closely similar to mammalian α-MSH (Table 1: 2, 4, 5). A persistent tyrosine contaminant at the level of 20 per cent of the other amino-acids was noted. (This work will be reported in detail later.)

Assay showed the specific activity of dogfish peptides PI and PII to be lower than that of mammalian MSH. This is in agreement with the results obtained with a synthetic peptide of similar composition[21]. Although PI could be an artefact introduced during extraction, we think that this is unlikely in the mild conditions used. The presence of PI and PII in similar proportions may simply demonstrate the parallel occurrence of two active pituitary peptides or may suggest that amidation of the COOH terminus confers some protection on the peptide after it has been released from the gland. The melanin dispersing ability of these preparations in elasmobranchs was confirmed by injecting the pure peptides into pale

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Table 1. PARTIAL AND COMPLETE AMINO-ACID SEQUENCES OF ADENOHYPOPHYSIAL AND SYNTHETIC PEPTIDES WHICH HAVE MELANOCYTE STIMULATING ACTIVITY

1	H Ser Tyr Ser Met Glu His Phe Arg Trp Gly Lys Pro Val Gly Lys Lys Arg . . . Phe OH^{39}
2	Acetyl Ser Tyr Ser Met Glu His Phe Arg Trp Gly Lys Pro Val NH_2
3	H Ser Met Glu His Phe Arg Trp Gly Lys Pro Val OH
4	H Ser Met Glu His Phe Arg Trp Gly Lys Pro Met OH
5	H Ser Met Glu His Phe Arg Trp Gly Lys Pro Met NH_2
6	H Met Glu His Phe Arg Trp Gly OH
7	H His Phe Arg Trp Gly OH
8	H Asp Ser Gly Pro Tyr Lys Met Glu His Phe Arg Trp Gly Ser Pro Pro Lys Asp OH
9	H Ala Glu Lys Lys Asp Glu Gly Pro Tyr Arg Met Glu His Phe Arg Trp Gly Ser Pro Pro Lys Asp OH
10	H Glu . . . Ala Ala Glu Lys Lys Asp Ser Gly Pro Tyr Lys Met Glu His Phe Arg Trp Gly Ser Pro Pro Lys Asp NH_2
11	H Glu . . . Ala Ala Glu Lys Lys Asp Ser Gly Pro Tyr Lys Met Glu His Phe Arg Trp Gly Ser Pro Pro Lys Asp^{58} Lys Arg . . . Glu NH_2^{90}

1, Corticotrophin; 2, α-MSH; 3, synthetic; 4, dogfish MSH PI; 5, dogfish MSH PII; 6, synthetic; 7, synthetic; 8, ovine β-ser^2-MSH; 9, human β-MSH 10, ovine γ-LPH; 11, ovine β-LPH.

skate from which the neuro-intermediate lobe of the pituitary had been removed. The fish rapidly darkened.

α-MSH and β-MSH were also isolated from ox pituitaries by the chromatographic methods we have described. The fractionation patterns of α-MSH and dogfish MSH were in agreement with their structural similarities, but no counterpart for β-MSH was found and we concluded that it is absent from the dogfish pituitary gland. Biologically active peptides with chromatographic and electrophoretic characteristics in common with β-MSH have recently been reported in selachians, holocephalans and teleosteans, but these studies were not carried out on pure peptides[22]. Geschwind[6] reported the presence in the pituitary gland of the teleost *Gadus* of two biologically active components with electrophoretic mobilities similar to mammalian α-MSH and β-MSH, the former being the more abundant. Burgers[23] described electrophoretic separation of three biologically active components from pituitary extracts of the amphibian *Rana*—frog—and the reptile *Anolis*, none of which seemed to be identical with the mammalian hormones.

Several Types of MSH

The presence in a single pituitary of several hormones with melanocyte-stimulating activity (up to seven in mammals if the reported occurrence of two corticotrophins and two β-MSHs is a general phenomenon) raises the question of whether they exist *in vivo* as separate entities, or result from the partial breakdown of parent molecules during preparative procedures. The species differences in particular hormones, coupled with the lack of knowledge of a complete series of amino-acid sequences for all the hormones of any one species, make it impossible to decide whether this is possible, but a comparison of the available sequences is nevertheless interesting. The common element is the heptapeptide "core": methionyl glutamyl histidyl, phenylalanyl arginyl tryptophanyl glycine, a sequence which by itself has considerable MSH activity (Table 2). The pentapeptide his.phe.arg.trp.gly. has also been reported to have MSH activity[20], but this may be too low to have any biological significance. The differences between the hormones consist partly of substitutions of one amino-acid for another, which is also the usual form of species differences in a given hormone, and partly of the lengthening of the amino-acid chain on either or both sides of the core. The core is roughly in the middle in the short chains of α-MSH and β-MSH and at the amino end of the ACTH molecule. β-MSH is an uncoiled polypeptide chain and ACTH is a random coil with no secondary or tertiary structure[24], although recent reports of the spatial conformation of the insulin molecule show that even a relatively small peptide can have a complex tertiary structure[25].

Table 2. MELANOCYTE STIMULATING ACTIVITY OF NATURALLY OCCURRING AND SYNTHETIC PEPTIDES

No.	Peptide	Relative potency
1	Corticotrophin	1×10^4
2	α-MSH	1×10^6
3	Synthetic	1×10^4
4	Dogfish MSH PI	1×10^4
5	Dogfish MSH PII	1×10^4
6	Synthetic	1×10^3
7	Synthetic	1
8	Ovine β-ser^2-MSH	1×10^5
9	Human β-MSH	1×10^5
10	Ovine γ-LPH	1×10^3
11	Ovine β-LPH	1×10^3

The numbers refer to sequences listed in Table 1. It is doubtful whether more precise estimates of potency are useful in view of the widely different methods of assay which have been used in obtaining the figures quoted.

This table shows approximate potency relative to the shortest synthetic peptide with MSH activity.

Comparison of Sequences

The position of the core relative to the terminal amino-acids suggests that certain linkages in conjectural parent molecules are more readily broken than others. The peptides fall naturally into two divisions, the members of each having common points of similarity and difference in comparison with members of the other division. Thus in Table 1 peptides 1 to 5 form one group, including the naturally occurring hormones ACTH, α-MSH and the dogfish MSHs PI and PII; and peptides 8 to 11 form a second group which includes β-MSH, β-LPH and γ-LPH. The peptides in the first group have the amino-acid serine immediately before the common heptapeptide and lysine immediately after, a situation which is exactly reversed in the second group. In both groups the next residue at the carboxyl end is proline and no species differences have been found at this point. The carboxyl ends of the second group of peptides are longer than in the first group, except for ACTH, and are almost identical for the whole of β-MSH and γ-LPH and up to residue 58 in β-LPH. In ACTH the amino-acid chain is extended considerably beyond the common element and the lysine and arginine at positions 16 and 17 in corticotrophin are found in β-LPH in the same place relative to the core, that is, positions 59 and 60. The dogfish MSHs, α-MSH and corticotrophin are all similar on the amino side of the core and the chain is shorter than in the second group of peptides. β-ser^2-MSH, present in the sheep, is identical with the sequence 41 to 58 in sheep γ-LPH and sheep β-LPH, and all the second group peptides are closely similar over the first six to ten residues on this side of the core. Although human β-MSH has two amino-acid substitutions in this region which are not found in non-primate β-MSHs, the four residues of its extended NH_2 terminus are identical with those in the same positions relative to the core in the LPHs isolated from the sheep (Table 1).

The isoelectric points of the β-MSHs studied so far lie between *p*H 5·5 and 7·0. The charge on the hormone at physiological *p*H varies according to the amino-acid substitutions present. Serine or glutamic acid may be present at position 2 in ovine β-MSH, affecting not only its charge but possibly also its potency, as β-glu^2-MSH is reported to be more than twice as potent as β-ser^2-MSH or the similarly charged equine β-glu^2, arg^{16}-MSH when assayed *in vitro*[6]. α-MSH is more basic, having an isoelectric point between *p*H 10·5 and 11·0. Burgers[23] reported that the major biologically active MSH component in codfish pituitary glands has an electrophoretic mobility consistent with that of α-MSH, and Geschwind[6] reported that the minor component was probably more basic than β-glu^2-MSH. The isoelectric points of the dogfish MSH peptides were calculated to lie between *p*H 10·5 and 11·5.

Structure and Activity

Table 2 shows the approximate relative potencies of the hormones and of some synthetic peptides. (This subject has been reviewed by Harris[26] and Geschwind[6].) With regard to the effects of structural differences, it is sufficient to point out that many changes which can be construed as protecting the core from enzymatic breakdown enhance biological potency. For example, acetylation of the NH_2 terminus of a synthetic peptide which is closely similar to dogfish MSH (No. 3 in Table 1) increased its MSH activity twenty-five-fold[21]. α-MSH has the greatest specific activity of all naturally occurring peptides and a lengthening of the polypeptide chain at either end, as in ACTH, on the one hand, and β-MSH on the other, results in a reduction in biological potency. Nevertheless it must be remembered that biological potencies can vary widely for the same molecule measured *in vivo* and *in vitro*, and that because very little is known about how MSH acts at the cellular level there remains the possibility that some differences in potency may be attributable to different effects on the target organ.

At the other end of the spectrum of biological activities is the stimulation of the adrenal cortex, which is primarily the function of ACTH and only exhibited to a much smaller extent by LPH and MSH. Because it is known that a synthetic peptide identical with the first twenty-three residues of ACTH possesses full biological potency, residues 24–39 cannot be one of the molecular groupings responsible for this action. It is interesting that interspecies variation in this hormone, so far investigated only in mammals, is found in residues 25–32. And so it seems that residues 14–23, probably acting in conjunction with residues 1–13, are responsible for the adrenocorticotrophic effects. Synthetic peptides identical with residues 1–17 and 1–19 possess some adrenocorticotrophic activity[24]. The dipeptide lys.arg. occurring at positions 16 and 17 in ACTH is also found at positions 59 and 60 in β-LPH and might be responsible for the low ACTH activity reported for the latter hormone[6]. However, γ-LPH, which stops short at residue 58, also has significant corticotrophic activity, as does MSH. The possibility that some of the preparations were contaminated with small amounts of ACTH and that this is at least in part responsible for the results cannot be ruled out. The lipolytic potency of the hormones, as exhibited in adipose tissue of rat and rabbit, is strongest in γ-LPH and β-LPH, although both ACTH and MSH seem to possess some activity[7]. At this time it is impossible to indicate any particular groupings responsible for this action. The effect is presumably due to changes in the concentration of tissue lipase, which is also affected by many other agents including growth hormone, glucagon and the catecholamines. The mechanism responsible is not understood at all. LPH has not so far been identified in the pituitaries of non-mammalian species.

The physiology and chemistry of MSH, ACTH and LPH encourage speculation on several evolutionary questions. Although elasmobranchs are not in the direct line of vertebrate ascent, the close similarity between dogfish MSH and α-MSH on the one hand, and between α-MSH and mammalian ACTH on the other, indicates certain features that would be expected in the ACTH of elasmobranchs, which has been found in the dogfish[27]. A foreshortened amino terminus of H-ser. or possibly H-tyr.-ser. is likely and a met.-val. substitution might be expected at residue 11. A shorter carboxyl end is also possible in view of the ACTH activity of the synthetic analogues. Other points of more general interest are, first, is α-MSH more primitive than β-MSH ?; second, what are the primitive functions and inter-relationships of the peptides with MSH activity ?; and third, how has MSH evolved ?

Is α-MSH Primitive ?

α-MSH is not only the most highly potent of the MSHs in causing the dispersion of melanin, it is also the shorter of the two naturally occurring forms. Its high potency probably results from the possession of an acetyl.ser.tyr.ser. terminal tripeptide which protects the biologically active core from enzymatic degradation and yet does not "bury" it further inside the molecule than is necessary for optimum effectiveness. The high potency of α-MSH, coupled with the fact that it is found unchanged in all mammals so far investigated, suggests that it may be the more primitive melanin dispersing hormone. Our work on dogfish MSH strongly supports this concept. Not only is the dogfish MSH we isolated basically similar to α-MSH, it is actually two residues shorter, which suggests primitiveness. It is the shortest MSH so far extracted as the principal biologically active peptide from any organism. The relationship of the serine and lysine on either side of the heptapeptide core is of the α-MSH type and the tyrosine contaminant observed, coupled with the end group and sequence analysis results, suggests that a certain proportion of molecules may have the sequence tyr.ser.met. and so on. At the carboxyl end there is a simple substitution of methionine for valine.

There can be little doubt of the common origin of α-MSH and dogfish MSH—one form of the latter is even amidated at its carboxyl terminus. But our procedures failed to demonstrate the presence of any β-MSH-like peptides in dogfish pituitaries although both α-MSH and β-MSH were clearly demonstrable in extracts of ox pituitaries using the same preparative methods. Whether or not this observation also indicates the primitiveness of α-MSH awaits further elucidation of the fish MSH peptides mentioned earlier.

The close similarity of the β-MSHs to the LPHs and the absence of blocking radicles at the NH_2 and COOH termini suggest that β-MSH might result from partial degradation of LPH. This idea is supported by the fact that the β-MSHs end with aspartic acid, for this amino-acid is in the same position relative to the heptapeptide core, that is, at position 58, in both β-LPH and γ-LPH. γ-LPH

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is identical to a subfraction of β-LPH, cleaved after residue 58, which suggests that the asp.-lys. bond at this point might be particularly labile or susceptible to specific enzyme cleavage. A lys.-asp. cleavage at the other end of the peptide could also account for all the non-primate β-MSHs so far reported, by the splitting of their respective lipotrophins if these exist with the corresponding specific amino-acid substitutions. By the same argument the extra tetrapeptide at the NH_2 terminus of human β-MSH would result either from the greater lability of bond 36–37 in human LPH or the existence of a different enzyme system. Cytological evidence regarding the pituitary origin of the two hormones does not, however, support this concept and more LPH sequences are needed before any conclusion can be drawn.

Primitive MSH Peptides

The cyclostomes, the most primitive living vertebrates, can change colour in the wild, and hypophysectomy of the lamprey results in the aggregation of melanin so that the animal becomes pale. Thus the ability to disperse melanin is of great antiquity in vertebrates. Protective coloration and an ability to change colour to merge with the background probably had survival value in the sluggish heavily armoured agnathan ancestors of vertebrates. It must be pointed out that adrenal cortex-like tissue is present in cyclostomes and that mobilization and depletion of body fat reserves also occur at certain seasons in many fishes. ACTH function and LPH function may already be established in the primitive vertebrates. But although the chemistry of the principles responsible for these activities in primitive forms awaits elucidation, the available information supports the hypothesis that dispersion of melanin is the most primitive attribute of the three. Thus the peptide "core" common to all has substantial MSH activity but must be deficient in the other properties. Progressive extensions and substitutions may have been responsible for the evolution of the two divergent groups, ACTH and α-MSH on the one hand, and β-MSH and LPH on the other. A second possible alternative is the occurrence of a large molecule with all three types of activity. The level of secretion and its duration could determine the stimulation of the appropriate target tissue.

Although the role of MSH in mammals is not fully understood, the preservation of the heptapeptide core, coupled with the apparently random variations at certain other sites in the mammalian β-MSHs, suggests that the core sequence is important in the molecule itself and that the molecule is important to the animal. It seems inconceivable that the genetic code for this particular sequence could have been derived and preserved on more than one occasion in a group of obviously related hormones. It is perhaps significant that melanocytes originate in the cells of the neural crest of the embryo and that neurological effects have been reported for both MSH and ACTH in experiments on mammals[28,29].

The Question of Evolution

If α-MSH and dogfish MSH derive from a biologically active molecule present in a common ancestor, it probably had either methionine or valine at the COOH terminus. The substitution of one hydrophobic amino-acid for another would not affect biological potency. A single mutational nucleotide base change is sufficient to account for the substitution. The amidation of the COOH terminus of α-MSH is also found in a proportion of dogfish MSH and must result in the latter in differing degrees of susceptibility to carboxypeptidases. On the amino side of the heptapeptide core serine is present in both cases, and in a high proportion of dogfish MSH it is at the NH_2 terminus. But, as we have noted, as much as 20 per cent of the dogfish hormone may have the sequence H tyr.ser. met. . . ., which clearly approaches the Acetyl.ser.tyr.ser. met. . . . of α-MSH. Whether or not the situation in the present day dogfish represents an early stage in the evolution of the mammalian peptide, the primitive elasmobranchs diverging as they did more than 300 million years ago from the line of evolution leading to mammals, we know that elongation of this peptide brings about a progressive increase in potency which selection might favour. However, although acetylation of dogfish MSH would bring about a twenty-five-fold increase in potency, this has apparently not happened, which suggests that the melanocyte physiology in the dogfish may require a more labile hormone.

If evolutionary changes leading to increase in the length of peptide chains occur by mutational elongation of the specific DNA, the addition of tyrosine at the NH_2 terminus of this peptide could be accounted for by the replication of the appropriate serine codon followed by a single base substitution, or by a defect in genetic duplication which resulted in an initial "out of phase" rendering of the code, followed at once by the restart of the correct sequence. It is easier to derive the first stages of the β-MSH/LPH sequence from the heptapeptide core than the α-MSH/ACTH pattern. Thus duplications of the appropriate codons followed by single base substitutions will yield lysine or arginine but not serine from methionine at the NH_2 terminus and serine but not lysine from glycine at the COOH terminus.

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New Hominid Remains and Early Artefacts from Northern Kenya

Five hominid specimens, including two well preserved crania, early artefacts and a rich collection of vertebrates have been recovered from Plio-Pleistocene deposits near Lake Rudolf. Some of the artefacts were located *in situ* in a horizon that has since been reliably dated with a new isotopic technique at 2·61±0·26 million years. They constitute the oldest known *in situ* dated unequivocal artefacts.

Fauna and Artefacts from a New Plio-Pleistocene Locality near Lake Rudolf in Kenya

In 1968 the National Museums of Kenya organized a three month expedition into the area east of Lake Rudolf in northern Kenya (see Fig. 1), and a second three months were spent there in the summer of 1969. Both expeditions were greatly assisted by the Kenya Government.

Before 1968 no expedition had penetrated the region and little was known about its geology. Aerial reconnaissance in 1967 had, however, revealed extensive sediments, and so plans were made to survey the area in more detail. Miss A. K. Behrensmeyer of Harvard University joined the expedition in 1969 and her preliminary report on the geology of the region east of the Koobi Fora peninsula follows.

Fossil vertebrates are abundant throughout the sediments in the Koobi Fora area. A small collection was made and this is now housed in the National Museum, Nairobi, where more detailed studies are in progress. The collection included five hominid specimens. The brief description here of this material is to be followed later by a more detailed report by Professor J. Mungai of the University College, Nairobi. Artefacts were recovered from the surface in several places and at one site they were located *in situ* in a horizon that has since been dated by Fitch and Miller, using K–Ar techniques, at 2·61±0·62 m.y. which is Plio-Pleistocene age (see p. 226 of this issue). A full description of the artefacts is given by Dr Mary Leakey in an accompanying letter.

The vertebrate material has some similarities with that collected in the Omo Valley by the 1967 International Palaeontological Expedition[1], and detailed comparisons between the collections should prove interesting. The fauna includes the fish *Clarias* and *Polypterus* and the reptiles *Crocodilus niloticus*, *Euthecodon brumpti* and chelonians. Several genera and species of mammal were found, including some that are abundant in the Omo Valley beds. *Elephas* cf. *recki* is the most common proboscidean, *Deinotherium* being rare. Specimens of a very primitive elephant that closely resembles the material from the Lothagam Hill site near Lake Rudolf[2] were recovered from site *KF–I* and this material is consistent with the occurrence of the suid *Nyanzachoerus* at the same level. *Equus* and *Stylohipparion* are both present although they are not common. *Ceratotherium simum efficax* is better represented than *Diceros bicornis*. Several species of Hippopotamidae were collected, including both the tetraprotodont and hexaprotodont varieties. Suidae are common, those identified being *Nyanzachoerus* sp. (from *KF–I*), *Mesochoerus limnetes*, *Pronotochoerus*, *Notochoerus*, *Afrochoerus* and *Gerontochoerus*. *Mesochoerus limnetes* is represented by two complete and well preserved skulls. Giraffidae are rare and only a few fragments, possibly *Giraffa gracilis*, *Libytherium* and *Okapia*, were collected. Alcelaphini, Antilopini, Reduncini and Tragelaphini are common. At site *KF–I*, a bovine skull with horn cores resembling the Eurasian *Leptobos* was found. Another bovid of particular interest resembles *Lunatoceras* material from southern Africa. It is represented by one skull with horn cores and a single horn core.

Carnivora are quite common but most of the material is very fragmentary. A machairodont cranium with one side of the mandible intact was collected, but specific identification is not yet possible. *Felis* and *Panthera* are represented in the collection and one mandibular fragment of *Crocuta* was recovered. We have one representative of Mustelidae and this is very similar to, although larger than, the present day genus *Mellivora*.

Primate material is common in certain localities and 127 specimens were collected including some post-cranial remains. *Papio*, *Simopithecus*, *Colobus*, *Cercopithecus* and one specimen of *Galago* have been identified in a preliminary examination of the collection. A more detailed description of this material will be given by Dr Meave G. Epps and myself in a paper on the classification of the East African cercopithecoids which we are currently preparing.

Among the five hominid specimens are two well preserved and nearly complete crania. One of the crania (*FS*–158) resembles *Australopithecus boisei* from Olduvai. The specimen (see Fig. 2) lacks teeth but is otherwise complete. It was recovered by Dr Epps and myself from the lower part of *KF–IIA*, a horizon below the tuff that provided the isotopic date of 2·61±0·26 m.y. Three

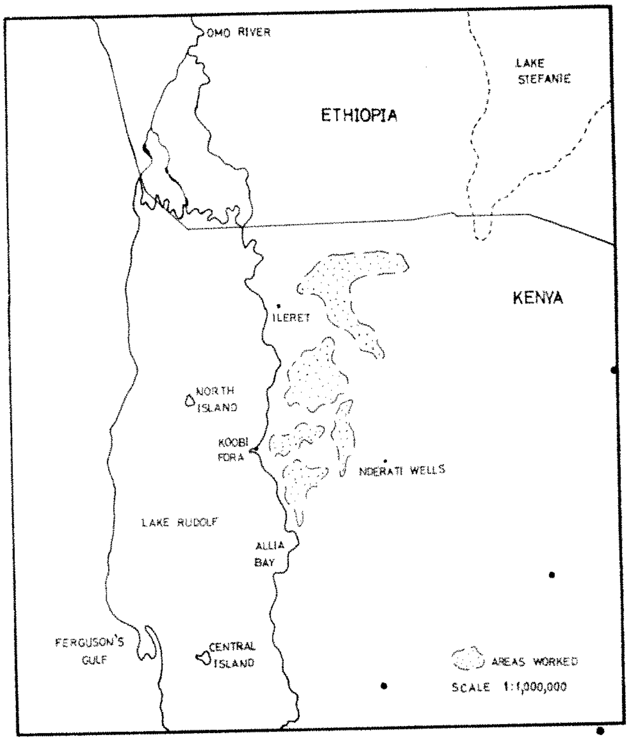

Fig. 1. Map showing the extent of the investigations in the Koobi Fora area, east of Lake Rudolf.

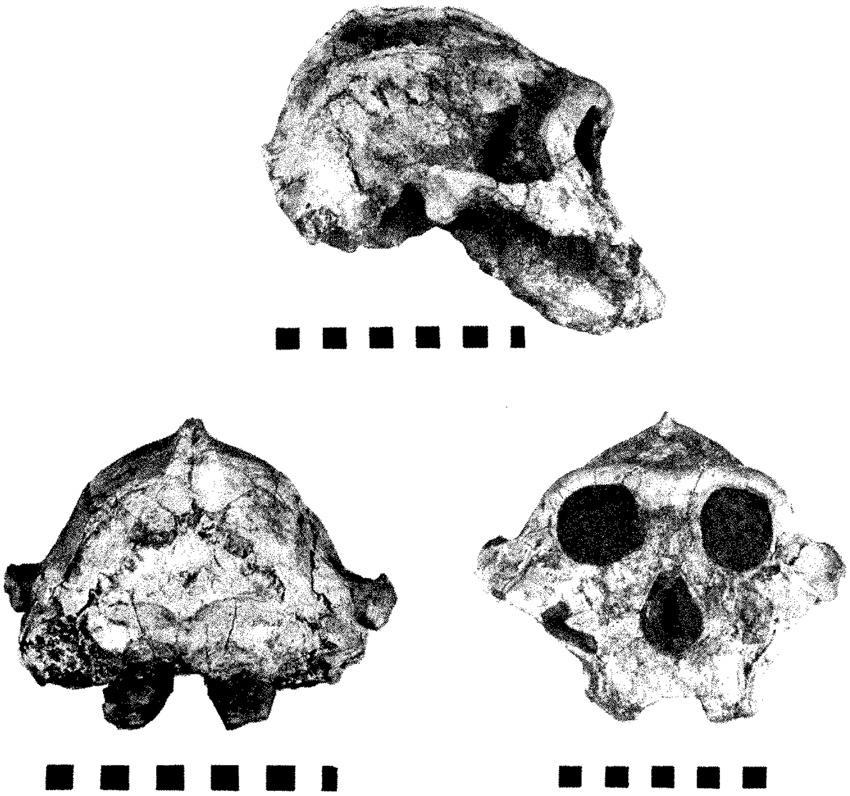

Fig. 2. Various views of *FS*–158—*Australopithecus boisei.*

other specimens, two mandibular fragments and an extremely weathered maxilla, were collected during the 1968 season, and these with *FS*–158 will be described by Professor Mungai. The four specimens may be conspecific. The second cranium (*FS*–210) was collected from a site approximately one mile north of the site where *FS*–158 was found, and was likewise from the lower part of *KF–IIA*. The specimen is fragmentary and only the basi-occipital region and the parietals are present (see Fig. 3). Its morphology contrasts markedly with ***Australopithecus boisei*** and it probably is either a gracile species of *Australopithecus* or else a very early representative of *Homo*; the latter possibility is considered more likely from the evidence of the occipital region, the temporals and the tympanic plate. It is hoped that further material will be recovered this summer. The discovery of *FS*–210 which seems to be distinct from the australopithecine *FS*–158 is consistent with the evidence from Omo and Olduvai in so far as more than one form of early hominid has been reported from both these East African localities. In view of the generally accepted opinion that *A. boisei* was not a toolmaker, the discovery of artefacts in the Koobi Fora deposits adds support to the evidence from the fossil material that there were two different representatives of hominids in the area we investigated.

All the artefact sites occur in the upper part of the tuff in *KF–IIA* and, in most cases, bone fragments were found in association with a few flakes and the occasional finished implement. In view of the significance of these living sites, I excavated a small trial step trench, 15 feet long by 4 feet wide at site *KBS*, to see if I could determine the exact stratigraphic position of the artefacts. Five flakes and fourteen bone fragments were uncovered and collected. The depth of the trench through the three steps was just less than 1 m and the consolidated and undisturbed nature of the matrix above the specimens confirmed that the material was *in situ*. The flakes that were recovered from the excavation were struck from the same raw material as the specimens lying on the surface of the site. The discovery of artefacts *in situ* in a horizon that has been dated at 2·61 ± 0·26 m.y. is very significant and further investigations in the Koobi Fora area are planned for this coming season.

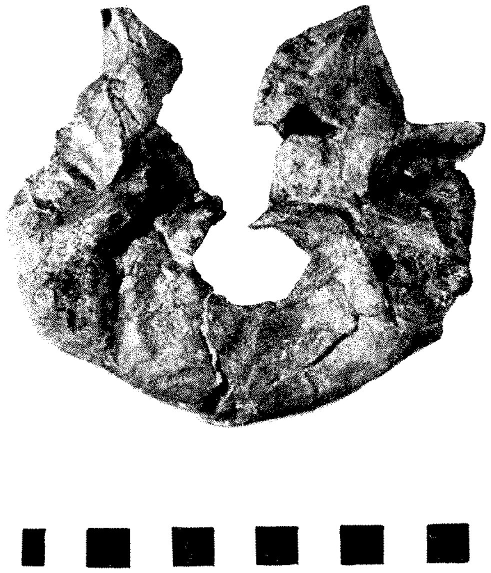

Fig. 3. Basal view of *FS*–210.

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Preliminary Geological Interpretation of a New Hominid Site in the Lake Rudolf Basin

A GEOLOGICAL reconnaissance of an area that included approximately 650 square miles between 3° 40′ and 4° 20′ north latitude and from the east shore of Lake Rudolf to about 36° 25′ east longitude was carried out during 1969. Within this area, a more detailed study was made of the exposures along an erosional escarpment east of the Koobi Fora peninsula (see Fig. 1 of previous report). This provided a shallow east–west cross-section of part of the eastern border of the Lake Rudolf Basin.

The sedimentary rocks of the region are generally low-lying, with a maximum relief of about 100 m above the present lake level, which is 370 m above sea level[1]. The deposits, of undetermined thickness but probably exceeding 300 m, occur throughout the area, and the oldest identified so far contain an early Pleistocene mammalian fauna. Overlying them unconformably are horizontal, sub-recent lake sediments. An extensive area of basalt flows and intrusions occurs in a broad ridge south-east of Ileret that roughly divides the sedimentary exposures into northern and southern parts. No flows were observed interbedded with the Pleistocene deposits, and the relationship of the volcanics to the fossiliferous sediments has not yet been clarified. Lenses of bentonitic ash-fall and water-deposited tuff occur throughout the sedimentary sequence. Several of these contain fresh pumice suitable for K–Ar dating.

The structural characteristics of the area seem to be closely associated with the tectonic setting of the East African Rift System[2]. A fault zone in the southern part cuts across the exposures of sedimentary rocks at Koobi Fora. There are no continuous major faults, but rather series of parallel fractures 0·5 to 3 miles long. These are generally oriented north–south, with individual throws of less than 50 m. All faulting seems normal, and the downthrown sides are usually on the west. The area within about 8 miles of the lake is the most heavily faulted, and parts of the section are often repeated or missing. Here the sediments are also folded in a broad anticline with a roughly east–west axis and an eastward plunge. The thickest continuous section, 250 to 300 m, is exposed in the anticline. The lower 100 m of this can be traced towards the east, where the bedding becomes horizontal, and can also be picked up north of the basalt ridge. In both places the upper part of the section is missing due to erosion or non-deposition.

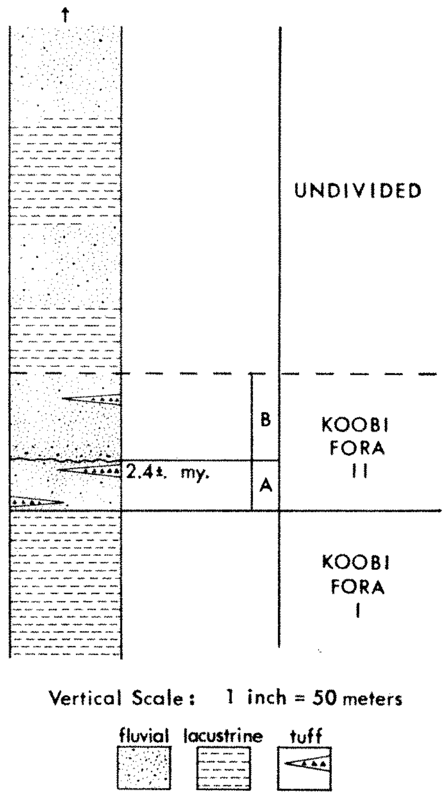

Fig. 2. Generalized stratigraphic divisions of the lower part of the Pleistocene sediments exposed at Koobi Fora. The predominant environment of deposition is indicated by the lithological symbols.

The Pleistocene sediments range from coarse sands and pebble conglomerates to fine silts and clays. Particle size, sedimentary structures, and the included fauna indicate deposition in at least two major types of environments; fluvial and lacustrine. A distinct lacustrine to fluvial transition occurs in the lower part of the section. Thinly bedded limonitic clays and sands are overlain by non-limonitic sandy clays, quartz and limonite pebble conglomerates and coarse cross-bedded sandstones. The change in lithology is easily recognizable and serves as the boundary between the lower lacustrine unit, designated as Koobi Fora I (*KF–I*), and the overlying fluvial unit,

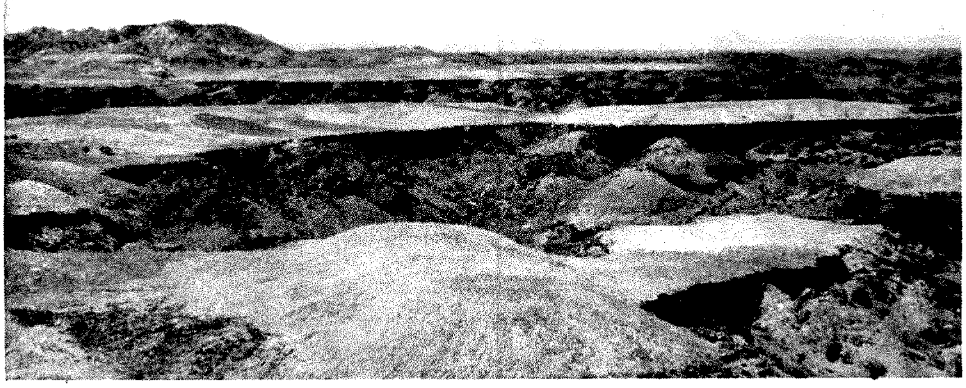

Fig. 1. Pleistocene exposures about 15 miles ENE of the Koobi Fora peninsula, looking east, with the mountains Shin and Nderati in the distant background. The prominent resistant bed marks the boundary between *KF–I* (lower, darker deposits) and *KF–II* (overlying, light-coloured deposits).

Koobi Fora II (*KF–II*) (see Figs. 1 and 2). *KF–I* is characterized by a predominantly fish and mollusc fauna, while *KF–II* is rich in mammals. *KF–II* is divided into a lower "*A*" and an upper "*B*" in the eastern part of the area by an erosional unconformity some 20 m above the base of the unit. The tuff bed containing stone tools *in situ* occurs high in *KF–IIA*. A preliminary evaluation of the fauna indicates that the time break represented by the unconformity is probably not significant. The lithology and stratigraphic sequences in *KF–II* are laterally persistent. On the basis of the almost identical stratigraphic successions and lithologies north and south of the basalt ridge, the sites of the hominid skull finds in the northern part are provisionally placed in *KF–IIA*.

The upper boundary of *KF–II* is tentatively set at the top of coarse quartz sandstones and conglomerates which cap the horizontal section east of the anticline. The thick section overlying *KF–II* has been studied only in the anticline and is not yet subdivided. It includes several alternating phases of coarse and fine sediments. Vertebrate and invertebrate fossils occur throughout, with mammalian remains more common in the coarser deposits.

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Radioisotopic Age Determinations of Lake Rudolf Artefact Site

SAMPLES of a volcanic tuff (Leakey *IA*) and of pumice lumps in a calcareous matrix (Leakey *II*) were sent to us by Mr Richard Leakey in July 1969 for an age determination feasibility study. Even if samples of pyroclastic rocks are completely fresh and otherwise suitable for potassium-argon age determination a number of specific additional sources of error may be present[1]. One of the most intractable of these is the possible presence of extraneous argon derived from inclusions of pre-existing rocks. Preliminary petrographic examination of the tuff sample revealed it to be a crystal–vitric–tuff rich in small pieces of pumice and minute crystal fragments set in a matrix of devitrifying volcanic dust. While much of this pyroclastic material was obviously juvenile, the presence of microcline suggested possible contamination from the wall rocks of the vent. The fine grain-size prevented effective separation of juvenile and possibly non-juvenile components, and an exploratory conventional total rock K–Ar age determination was made finally on the washed and mixed 30/50 mesh fraction of the crushed sample.

Six potassium oxide measurements were made by flame photometry and an average value calculated. Three independent argon isotope analyses were undertaken. Argon extractions and purifications were carried out in the manner described by J. A. M. and Brown[2]; isotope ratios were measured using an omegatron-type mass spectrometer[3]. Enriched argon-38 was used as an internal standard (spike). Errors in radiogenic argon volume arising from uncertainties in the isotopic ratios of the argon sample and spike volume, together with those introduced in the determination of potassium oxide content, are combined. The analytical error in millions of years associated with each separate age determination is calculated according to the method set out by us[4]. The results of these age determinations are given in Table 1. From these results it was clear that an extraneous argon age discrepancy was present, and that it would only be possible to date this tuff by careful extraction of undoubtedly juvenile components for analysis. The pumice lumps (Leakey *II*) would have been suitable if fresh, but on examination they were found to be intensively calcified. Minute sanidine inclusions in the pumice were promising, however, and in reporting the results of this feasibility study we suggested that a search be made of the tuff exposures in the field for (*a*) less calcified pumice lumps, and (*b*) sanidine phenocrysts that could be conveniently separated.

Table 1. ANALYTICAL RESULTS OF WHOLE ROCK TOTAL DEGASSING CONVENTIONAL K–Ar AGE DETERMINATIONS ON THE TUFF FROM *KF-IIA*

Sample	K_2O (per cent)	Atmospheric contamination (per cent)	v/m	Apparent age and error (m.y.)
Volcanic tuff	1·79	62·7	$1·37 \times 10^{-2}$	219 ± 7
(Leakey *IA*)	1·79	63·4	$1·40 \times 10^{-2}$	223 ± 7
	1·79	62·0	$1·39 \times 10^{-2}$	221 ± 7

$\lambda e = 0·584 \times 10^{-10}$ yr^{-1}; $\lambda\beta = 4·72 \times 10^{-10}$ yr^{-1}.
v/m = volume of radiogenic argon-40 (mm^3 NTP) per g of sample.

Further samples were received from East Africa in August 1969 and consisted of pumice lumps (Leakey *IB1*) and a collection of feldspar phenocrysts (Leakey *IB2*) separated from the tuff. The new pumice lumps, although strongly calcified in part, were considerably fresher than those in sample Leakey *II* and contained numerous fresh microphenocrysts of sanidine and aegerine–augite. The separated crystals were mostly of fresh sanidine, but did include a few aegerine–augite and composite feldspar–pyroxene–iron ore grains. Complete freedom from argon loss discrepancy cannot be expected at a site where carbonates have been so active in replacing volcanic glass, and in view of cracks observed in the separated feldspar crystals that may have occurred during explosive eruption, argon losses from the crystal fraction must be expected in addition to the possibility of extraneous argon errors arising from an admixture of non-juvenile crystals. Two exploratory conventional K–Ar age determinations were made to determine whether or not more detailed work on these new samples would be advantageous. One of these exploratory analyses was made on the core of a pumice lump, the other on the remainder of the crystal fraction after a number of the largest, most homogeneous and uncontaminated feldspar crystals had been picked out and held in reserve. These conventional analyses were carried out in a similar manner to that outlined above, and the results are given in Table 2.

Table 2. ANALYTICAL RESULTS OF EXPLORATORY CONVENTIONAL K–Ar AGE DETERMINATIONS ON FURTHER SAMPLES FROM THE TUFF FROM *KF-IIA*

Sample	Method	K_2O (per cent)	Atmospheric contamination (per cent)	v/m	Apparent age and error (m.y.)	Average apparent age and error (m.y.)
Leakey *IB1*	Whole	0·70	99·6	$8·49 \times 10^{-5}$	3·63 ± 2·1	
pumice	rock	0·70	91·1	$5·61 \times 10^{-5}$	2·40 ± 1·0	3·02 ± 1·6
Leakey *IB2*	"Feld-	6·04	81·2	$4·79 \times 10^{-4}$	2·38 ± 0·3	
crystals	spar"	6·04	80·5	$4·76 \times 10^{-4}$	2·36 ± 0·3	2·37 ± 0·3

$\lambda e = 0·584 \times 10^{-10}$ yr^{-1}; $\lambda\beta = 4·72 \times 10^{-10}$ yr^{-1}.
v/m = volume of radiogenic argon-40 (mm^3 NTP) per g of sample.

The two exploratory conventional age determinations were most encouraging. It was virtually certain that the pumice was juvenile and very likely that most, if not all, of the individual phenocrysts collected from the tuff were juvenile also. The proportionally large experimental error quoted with the apparent age from the pumice sample results from its low potassium content and the high value of atmospheric contamination. At this stage it was apparent that for satisfactory dating of the tuff horizon answers were required for the following three questions: (i) was the crystal fraction (Leakey *IB2*) entirely juvenile in origin ? (ii) were any argon losses or extraneous (that is to say, inherited or excess) argon discrepancies present ? and (iii) could the experimental errors be reduced ? In order to find answers to these questions two total degassing $^{40}Ar/^{39}Ar$ and two $^{40}Ar/^{39}Ar$ age spectra age determination analyses were undertaken. Description of the theory and practice of $^{40}Ar/^{39}Ar$ age determination can be found in Mitchell[5], Dunham *et al.*[6] and F. J. F. *et al.*[7]. $^{40}Ar/^{39}Ar$

geochronometry is based on the conversion of a portion of the natural isotope potassium-39 present in a rock or mineral sample to the artificial radioisotope argon-39 by neutron irradiation. The argon-39 yield for a particular neutron dose is proportional to the potassium content of the sample and a value for the constant of proportionality (J) can be derived from the argon-39 yield in standard samples irradiated by the same neutron dose. After an appropriate irradiation, measurement of the argon isotope ratios present in the sample and standard using a suitable high resolution mass spectrometer is all that is necessary for complete total degassing geochronometric analysis. The mass of the sample or standard does not enter into the calculation. Very small and/or inhomogeneous samples and samples of extremely low potassium content can be dated by this method.

^{40}Ar/^{39}Ar age spectrum geochronometry is a development of the ^{40}Ar/^{39}Ar dating technique in which the isotopic composition of the gas being released from an irradiated sample of the rock or mineral is analysed repeatedly over a series of heating steps towards complete fusion. When a rock or mineral sample of uniform age is analysed by this method a very accurate multipoint K–Ar isochron age is obtained. When a rock or mineral sample of mixed age is analysed by this method an "age spectrum" is obtained. This method can be a very powerful geochronological tool. It depends for its success on the fact that there are some argon locales in minerals from which argon can only be driven by extreme stimuli. Thus, if suitable material is chosen and the less tightly held argon removed first, it becomes possible to date the remaining "old" radiogenic argon by itself and to obtain some idea of the early history or crystallization date of the rock or mineral concerned. In practice, this technique frequently produces an age spectrum which can be directly related to the geological history of the rock and can be used to elucidate inherited and/or excess argon discrepancy. The results of the ^{40}Ar/^{39}Ar analyses undertaken on the second batch of samples from the *KF-IIA* tuff are given in Table 3.

Table 3. ANALYTICAL DATA FOR ^{40}Ar/^{39}Ar AGE DETERMINATIONS

(*a*) Total degassing ^{40}Ar/^{39}Ar age determinations

Sample	J used	Atmospheric contamination (per cent)	R	Apparent age and error (m.y.)
Leakey *IB1* pumice	$2·53 \times 10^{-2}$ (3)	97·9	$7·25 \times 10^{-2}$	3·45 ± 1·2
Leakey *IB2* feldspar	$2·63 \times 10^{-2}$ (4)	94·9	$5·45 \times 10^{-2}$	2·64 ± 0·29

(*b*) ^{40}Ar/^{39}Ar age spectrum on Leakey *IB2* feldspar

Run against standard No. (4) GG2 age 292 m.y. $Q = 0·16751$; $J = 2·63 \times 10^{-2}$. Each heating step 10 min.

Step	RF	Atmospheric contamination (per cent)	39/36	R	Apparent age and error (m.y.)
1	0	100	—	—	—
2	10	100	6·3	—	—
3	20	100	5·4	—	—
4	30	100	63·6	—	—
5	40	100	223	—	—
6	45	89·9	769	$3·35 \times 10^{-2}$	1·65 ± 0·16
7	50	85·3	1,047	$4·26 \times 10^{-2}$	2·13 ± 0·21
8	55	84·9	1,351	$5·25 \times 10^{-2}$	2·60 ± 0·26
9	60	84·0	1,505	$5·27 \times 10^{-2}$	2·61 ± 0·26
10	65	83·8	1,545	$5·26 \times 10^{-2}$	2·61 ± 0·26
11	70	85·2	1,432	$5·25 \times 10^{-2}$	2·60 ± 0·26
12	75	91·1	1,150	$4·87 \times 10^{-2}$	2·42 ± 0·24
13 (fused)	80	92·1	840	$2·80 \times 10^{-2}$	1·37 ± 0·14

Average plateau age = 2·61 ± 0·26 m.y.

(*c*) Restricted ^{40}Ar/^{39}Ar step heating analysis of Leakey *IB1* pumice

Step	Atmospheric contamination (per cent)	R	Apparent age and error (m.y.)
RF0 for 20 min at step 1	100	—	—
RF80 for 20 min at step 2	96·2	$5·26 \times 10^{-2}$	2·50 ± 0·5

J data

GG2 standard muscovite $Q = 0·16751$

Sample	Atmospheric combination (per cent)	R	Q	J
GG2 (No. 4)	3·5	6·37	0·16751	$2·63 \times 10^{-2}$
GG2 (No. 3)	2·7	6·63	0·16751	$2·53 \times 10^{-2}$

Reactor loading

Stack I	Stack II	Stack III
Leakey *IB1* pumice	GG2 standard (3)	Leakey *IB1*
Leakey *IB2* crystals	GG2 standard (4)	Leakey *IB2*

The addition of the four ^{40}Ar/^{39}Ar age determinations to the conventional analyses already made enabled the three questions formulated earlier to be answered.

(i) Juvenile origin of the separated feldspar crystals. Repetition of apparent ages identical within the experimental errors from three different samples of the phenocrystic crystal fraction (that is, 2·37 ± 0·3 m.y. (conventional K–Ar); 2·64 ± 0·29 m.y. (total degassing ^{40}Ar/^{39}Ar) and a plateau age of 2·61 ± 0·26 m.y. (^{40}Ar/^{39}Ar age spectrum)) strongly suggests that this crystal fraction is homogeneously juvenile. When this coincidence is considered in relation to the three independent ages obtained from the pumice (that is, 3·02 ± 1·6 m.y. (conventional K–Ar); 3·45 ± 1·2 m.y. (total degassing ^{40}Ar/^{39}Ar) and 2·5 ± 0·5 m.y. (step heating ^{40}Ar/^{39}Ar): regarded as the best age for the pumice, see below), then it becomes almost certain that both the sanidine phenocryst fraction and the pumice lumps are entirely juvenile and are the products of the same volcanic eruptive phase. Inherited argon errors, such as were present in the analysis of the total tuff (Leakey *IA*), are therefore not present in the age determinations made on the second batch of samples.

(ii) Presence of excess argon and argon loss discrepancies. Pumice is a froth of volcanic glass and escaping juvenile volcanic gases. Juvenile volcanic gases may contain radiogenic argon-40 derived from the crust or upper mantle. Normally, appreciable amounts of this "excess argon" are not retained in volcanic rocks erupted on the land surface or in rocks which have cooled slowly, but very small quantities may be trapped in volcanic glasses that have been quenched rapidly. This small quantity of excess argon only becomes significant, however, in the analysis of very young volcanic glasses such as the Leakey *IB1* pumice. Because, in addition, the possible occurrence of a small argon loss discrepancy is suggested by the presence of carbonates partly replacing glass and infilling vesicles, the results of the two total degassing analyses made on the pumice (that is, 3·02 ± 1·6 m.y. (conventional K–Ar) and 3·45 ± 1·2 m.y. (total degassing ^{40}Ar/^{39}Ar)), each may contain two small inherent geological errors of opposing sign and of amounts that cannot be estimated (but are unlikely to be greater than the quoted experimental errors which are themselves large because of low potassium content and high atmospheric contamination). The possible presence of these unmeasured errors means that the best estimate of the age of the pumice which can be arrived at from the total degassing analyses would be that it lies somewhere between 2·25 and 4·62 m.y. (the total error span of the two determinations). Excess argon is not tightly held in volcanic glass; thus it is driven off at low temperatures during a ^{40}Ar/^{39}Ar age spectrum analysis. The pumice sample Leakey *IB1* was too young and potash-poor for a full age spectrum analysis, but it was possible to make a simple two-step analysis on it. All weakly held argon was driven off during a 20 min low temperature step-heating: the sample was then fused and the isotope ratios of the remaining argon measured. The apparent age obtained from this second step, 2·5 ± 0·5 m.y., can be regarded as a close minimum age for the pumice. It is most probably free of excess argon discrepancy, although not of argon loss discrepancy, and has a smaller experimental error because of the reduced atmospheric contamination.

It is unlikely that excess argon discrepancy is important in any of the crystal fraction age determinations, for this error would be insignificantly small when compared with the high potassium content of the samples. As was suggested earlier, minor variable argon loss discrepancy is likely to be present. The two total degassing analyses (that is, 2·37 ± 0·3 m.y. (conventional K–Ar) and 2·64 ± 0·29 m.y. (total degassing ^{40}Ar/^{39}Ar)) must therefore be

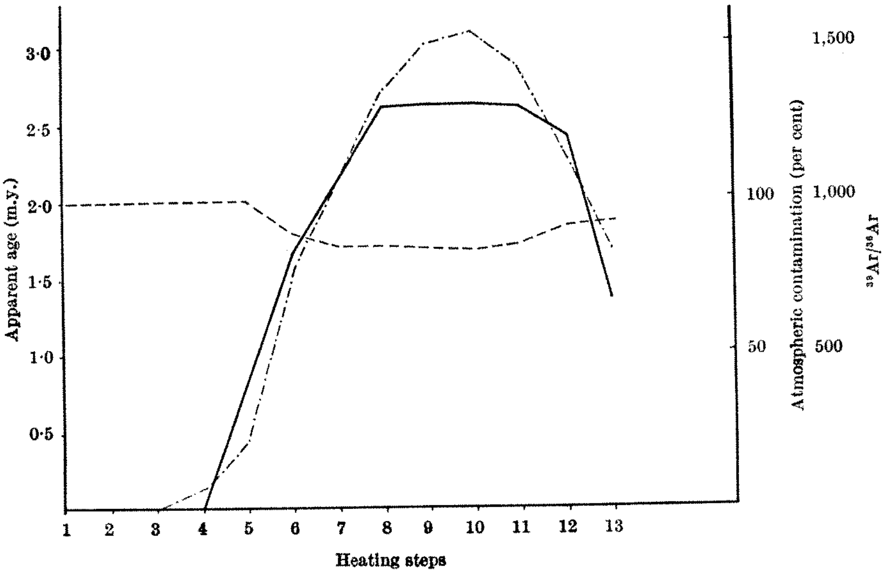

Fig. 1. ^{40}Ar/^{39}Ar age spectrum analysis of crystal fraction Leakey *IB2*. ———, Apparent age; - - -, atmospheric contamination; — · — · —, argon release.

suspect to some extent because of the possible presence of these unmeasured errors. Because the ^{40}Ar/^{39}Ar analysis was made on some of the best sanidine crystals selected from the sample provided, it is very probable that the argon loss discrepancy in this analysis is much less than in the conventional age determination which was made on the remainder of the crystal fraction.

Final resolution of the excess argon and argon loss problems was obtained from the ^{40}Ar/^{39}Ar age spectrum analysis of the best of the selected sanidine crystals (see Fig. 1). The nature of the age spectrum obtained proves that, in this sample, while excess argon discrepancy is at a minimum, a measurable amount of argon loss has occurred. The flat apparent age plateau obtained during the period of maximum argon release can be regarded, however, as a very close estimate of the true age of crystallization (that is, at 2·61 ± less than 0·26 m.y.).

Thus the age indications obtained in this survey can be summarized as follows:

Leakey *IB1* pumice: between 2·25 and 4·62 m.y.; 2·5 ± 0·5 m.y. is a close minimum age.

Leakey *IB2* crystals: 2·37 ± 0·3 m.y. is a minimum age; 2·64 ± 0·29 m.y. is a reasonably close age; and 2·61 ± less than 0·26 m.y. is a very close age estimate.

These age indications are all consistent. The best and most acceptable estimate is clearly 2·61 ± less than 0·26 m.y.

(iii) Reduction in experimental error. When the rock being dated is very young it becomes important to reduce the experimental errors to a minimum because they are frequently of a similar order of magnitude to that of the date required. The lowest errors are obtained when the potassium content of the sample is high and the atmospheric contamination is low. These conditions are best satisfied during the ^{40}Ar/^{39}Ar analyses of the selected feldspar crystals. The error on the accepted age of 2·61 m.y. is quoted as less than 0·26 m.y. because of the fourfold repetition of this apparent age and error as a plateau on the age spectrum. The overall indications of the results obtained here suggest that the real age of the tuff on the K–Ar time-scale must be very close to 2·6 m.y.

It can therefore be concluded that the best interpretation of the results of three conventional K–Ar age determinations and four ^{40}Ar/^{39}Ar age analyses on pumice, crystal and whole rock fractions of the tuff horizon in the Koobi Fora beds from east of Lake Rudolf in East Africa is that its age is very close to 2·6 m.y. (2·61 ± less than 0·26 m.y.). This is a Plio-Pleistocene age. In terms of the American succession it would seem to be equivalent to some part of the Blancan.

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Early Artefacts from the Koobi Fora Area

ARTEFACTS have been recovered from only a few localities in the Koobi Fora area and no locality has provided sufficient material for the nature of the industry to be assessed in full. The fifty-one specimens described here are the largest series from one site (*KBS*). They consist of five choppers, twenty-four flakes, two refresher flakes, two roughly flaked and battered cobbles, seventeen broken flakes and chips and one unmodified cobble. Five of the flakes were *in situ* in a fine-grained silty tuff, at a depth of just under 1 m from the top of the deposit. Fourteen fragments of mammalian bones, one porcupine tooth and a bovid molar were also found at the same level. As has been noted by R. E. F. Leakey in an accompanying report, the balance of the artefacts lay on the surface, close to the *in situ* flakes and bones, distributed in such a manner as to suggest that they had originally been embedded in the tuff and had weathered out during comparatively recent times. The material of the *in situ* and surface specimens is indistinguishable, and in view of the fact that comparable artefacts do not occur outside the immediate vicinity of the site, it seems justifiable to assume provisionally that the whole series is derived from the same source. Furthermore, the few similar artefacts that are known from other sites at Koobi Fora are also associated with this tuff, although no other locality has so far been excavated or yielded *in situ* material.

A few of the surface specimens are weathered, but most are in a remarkably fresh condition and clearly have not been exposed for any length of time, either since eroding from the tuff or before being buried in it—always assuming that they are contemporaneous.

The palaeogeography of the Koobi Fora area has not yet been studied in any detail. Preliminary observations, however, indicate that the artefact-bearing tuff fills depressions, most likely former erosion channels, in the underlying sediments. At the tool site, the tuff is fine-grained and silty, but it contains coarser elements elsewhere and the fresh pumice on which the isotopic age determinations have been carried out was obtained from a locality approximately 1 mile distant. More extensive excavation will be needed before it is possible to assess the nature of the tool site, but judged on similar artefact occurrences in Bed I at Olduvai, it seems most likely to have been a temporary camping place occupied for only a brief period.

One of the *in situ* flakes and one flake from the surface were submitted to Dr R. L. Hay, Department of Geology, University of California, Berkeley, who reports on the raw materials as follows.

"Both specimens are a trachyandesite or basalt, consisting largely of plagioclase (approximately An_{50} in composition), clinopyroxene (probably augite) and glass. Plagioclase is slightly more abundant than the clinopyroxene. The specimens are strikingly similar in texture and composition and could have come from the same flow. The lava, however, is of a type rather common in the Rift Valley, and I cannot rule out the possibility of different sources."

Choppers.—These consist of two classic choppers made on cobbles in which the working edges have been boldly flaked on both faces, one somewhat weathered double-edged specimen and two smaller and less typical examples. The first two specimens are in mint condition. One is a side chopper (*KBS* 4) with alternate multidirectional flaking. No chipping or other evidence of utilization is evident. The butt is rounded and consists of cortex surface (Fig. 1*A*). The second example (*KBS* 9) is also made on a cobble and is two-edged with a working edge at either end and a third sharp edge linking the two. The edges at either end are curved and more extensively flaked on one face than the other. They are slightly chipped, apparently by use, but are still jagged and sharp and have not become blunt (Fig. 1*B*). Specimen *KBS* 34 is also double-ended, but each working edge has been flaked from one direction only. Because of weathering, it is difficult to determine whether the flaking represents deliberate shaping or is merely the result of heavy utilization. Chopper *KBS* 15 is conical and has been trimmed round the circumference of the base. This consists of a single negative scar that has served as the striking platform (Fig. 1*D*). *KBS* 24 is the smallest chopper in the series and has a mean diameter of 34 mm. The working edge is trimmed by means of a single flake on one face and three flakes on the opposite face. The tool is slightly weathered and the butt consists of cortex surface (Fig. 1*C*). The measurements for the five choppers are given in Table 1.

Flakes.—Twenty-four of the flakes are complete. They are all irregular and mostly divergent, only five examples being convergent with the maximum width at the butt. In five specimens, an area of cortex surface is visible on the dorsal aspect, indicating that these flakes were struck from cobbles or boulders. The angles of the striking platforms can be measured in twenty-one specimens and range from 90° to 120° with an average of 108°. The edges of the flakes are particularly sharp and one example shows chipping that may have been caused by use. The bulbs of percussion are variable, but on the whole are well marked. End-struck flakes predominate and amount to 63 per cent of the total. They range in size from 62 × 41 mm to 13 × 12 mm with an average length/width measurement of 44 × 33 mm. Side-struck flakes range in size from 54 × 81 mm to 18 × 23 mm, with an average of 33 × 44 mm.

Table 1. MEASUREMENTS OF THE FIVE CHOPPERS

	Length	Width	Thickness	Mean diameter	Edge length	Number of scars
KBS 4	63 mm	74 mm	57 mm	64 mm	97 mm	6
KBS 9	68	54	51	57	45 and 51	4 and 7
KBS 15	38	47	35	40	135	8
KBS 24	35	39	28	34	31	3
KBS 34	48	51	35	45	52 and 45	?

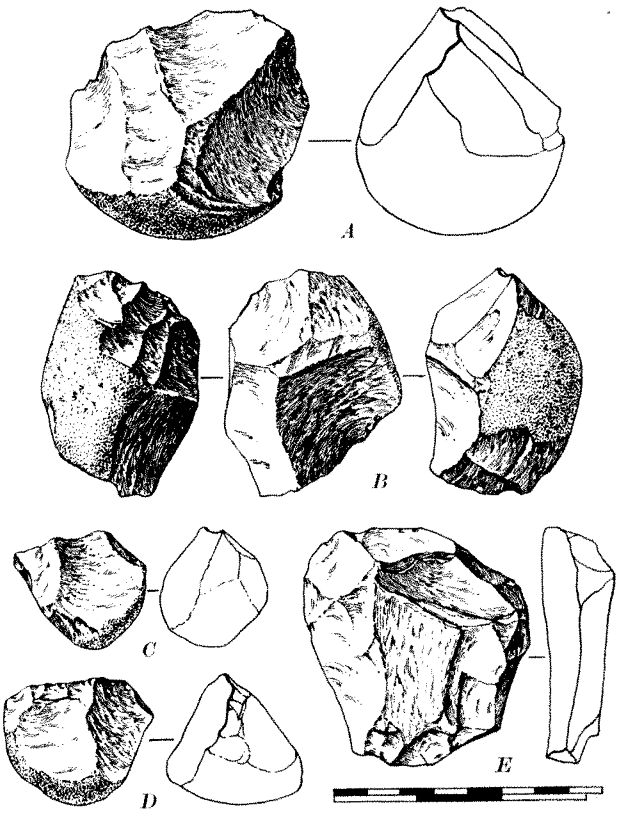

Fig. 1

Refresher Flakes.—Two flakes show the bifacially flaked crests that are characteristic of refresher flakes struck from cores and that also occur in the Oldowan on flakes that have been struck from choppers in order to re-sharpen the edges. In one specimen (Fig. 1*E*) the crest on the dorsal aspect extends along one lateral edge and then curves transversely across the width of the flake. In the second example, the crest is longitudinal and runs the whole length of the flake, from the butt to the tip. The measurements for these two flakes are 71 × 60 mm and 66 × 61 mm respectively.

Flaked and Battered Cobbles.—There are also two cobbles with indeterminate flaking and battering. One example (*KBS* 23) is considerably weathered. It has a number of negative scars and the intervening ridges are battered and crushed (86 × 64 × 53 mm). The second specimen is oblong and has crude bifacial flaking along one lateral edge as well as at one end (81 × 61 × 52 mm).

Until more *in situ* material has been obtained it is premature to comment on the elements that go to make up the artefact assemblage from Koobi Fora, or to draw parallels with the Oldowan and other early industries. It is surprising to note, however, that the technique of flaking seen on two of the choppers and on a proportion of the flakes appears to be bolder and more assured than the flaking usually seen on Oldowan tools. But it is possible that this anomaly may be due to the use of more homogeneous material in which it was possible to control with greater accuracy both the direction and the extent of fracture.

In conclusion, it can be stated that the evidence for artificially struck flakes in a tuff that has been dated at 2·6 ± 0·26 m.y. by Fitch and Miller (see their accompanying report) seems to be unquestionable. As regards the choppers and other specimens that were found on the surface, some element of doubt must remain until similar tools have been found *in situ*. But the balance of evidence indicates that these and possibly other artefacts from the Koobi Fora area are in fact derived from the tuff. If this is so, they antedate the earliest known artefacts from Bed I, Olduvai, by approximately 0·8 m.y. The few choppers found by L. S. B. Leakey at Kanam West are probably closer in age, but are not now available for comparison[1]. At Olduvai, the industry from sites immediately above the basalt that has been dated at 1·9 m.y. and that is overlain by a tuff for which an average date of 1·75 m.y. can be accepted, already contains a variety of recognizable tool types. There are several forms of choppers, polyhedrons, discoids, light and heavy duty scrapers and even a few burins. No significant evolution in the Oldowan is apparent throughout Bed I and the base of Bed II[2], a period that can be estimated to have lasted 0·4 or 0·5 m.y. The occurrence of choppers and other artefacts 0·8 m.y. before the earliest known Oldowan tools is therefore not unexpected. In fact, the multiple tool kit that was in use at Olduvai between 1·9 and 1·75 m.y. has led me to suggest that systematic tool making must have been in practice for a considerable period before that time[3].

I thank my son Richard for inviting me to describe the Koobi Fora artefacts and enabling me to visit the site where they were found, and Dr R. L. Hay for his report on the rock samples.

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Gene Order in the Bacteriophage R17 RNA: 5'–A Protein–Coat Protein–Synthetase-3'

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The sequencing of the ribosome binding sites and parts of the coat protein cistron has enabled the order of the three R17 genes to be established by direct chemical means. Translation may be influenced by the conformation of the RNA.

THE related coliphages R17, MS2, fr and f2 appear to encode only three proteins in their single-stranded RNA genome[1-4]. These are: (1) the coat protein, which is the major protein component of the virus particle (129 amino-acids[5,6]); (2) the maturation or A protein, a minor component of the virus particle (approximately 350 amino-acids[7]); and (3) the RNA replicase or synthetase, which is not present in the mature phage particle (a minimum of about 450 amino-acids[8]). The phage genome contains 3,300–3,500[9-11] nucleotide residues, of which the three cistrons account for approximately 2,800.

Conditional lethal mutants of these bacteriophages fall into three complementation groups,[1,2] which have been correlated with the three known phage proteins, but, because of the inability to obtain genetic recombination between these mutants, no genetic map of the phage has been constructed.

It is possible to detect the *in vitro* synthesis of the three phage proteins directed by the phage RNA[12-15]. This has led several groups to attempt the isolation of different forms and fragments of the RNA molecule, in order to determine their messenger activities and to infer the order of the cistrons along the RNA genome[14,16-21,25]. Their conclusions, however, are conflicting unless one admits the unlikely possibility that the closely related RNA bacteriophages have different gene orders. Moreover, all previously determined gene orders are subject to the criticism that they are based on indirect measurements and rely on various assumptions about the messenger properties *in vitro* of the RNA and its fragments.

The elucidation of the nucleotide sequences of the three ribosomal binding sites[22] and of parts of the coat protein cistron[23,24] has now made possible the determination of gene order by direct chemical means. On the basis of the distribution of these sequences between two specific fragments of the R17 RNA, we conclude that the gene order is: 5'-A protein—coat protein—synthetase-3'.

Preparation of Specific Fragments of R17 RNA

A partially purified endonuclease from *Escherichia coli* MRE600, referred to as ribonuclease IV, cleaves R17 RNA at a position about 40 per cent of the way into the molecule from the 5'-end[16,25,26]. Further cuts are introduced only slowly, permitting isolation of two specific fragments. *In vitro* translation studies in extracts from *E. coli* have shown that the 60 per cent fragment from the 3′-end directs efficient synthesis of the synthetase protein, while the 40 per cent fragment is relatively inactive as messenger[16,25]. The coat protein, which is the major product of *in vitro* protein synthesis with the native R17 RNA, is not efficiently produced by either fragment.

Investigation of the location of genes by RNA sequence methods requires ^{32}P-labelled fragments of high specific activity (about 10^6 c.p.m./μg). Because of radiation damage, RNA of such specific activity extracted from phage purified by conventional procedures is not intact, and ribonuclease IV cannot cleave it into clean fragments. By using chromatography on DEAE-cellulose for the initial step of phage purification (see legend to Fig. 1), however, damage can be minimized allowing isolation of intact RNA.

Treatment with ribonuclease IV as described[16,25,26] produces 15S and 22S fragments from the 26S native R17 RNA (Fig. 1). The 15S preparation represents the 5′ terminal 1,300–1,400 nucleotides (40 per cent) of the molecule[26]; its yield of the terminal pppGp residue[32] is nearly equivalent to that of the intact RNA (Table 1). This fragment must therefore be relatively free of material arising from other parts of the genome, as confirmed by its apparent homogeneity on polyacrylamide gels. The 22S RNA, containing 2,000–2,100 nucleotides (60 per cent)[26], comes from the 3′-end of the R17 RNA molecule; no more than 10 per cent of its chains terminate with pppGp (Table 1). On gels the 60 per cent preparation appears approximately 80 per cent homogeneous; its contaminants are smaller RNA fragments, some exhibiting the same mobility as the 40 per cent piece.

Table 1. ANALYSES OF 5′ TERMINAL pppGp

	Native RNA	60 per cent	40 per cent
Total c.p.m. ^{32}P	612,800	1,520,000	1,173,000
pppGp c.p.m.	487	302	2,180
Percentage ^{32}P in pppGp	0·079	0·020	0·186
Percentage P in pppGp (theoretical)	0·114	0·19	0·286
Percentage chains with pppGp	69	(10)	65

The pppGp analyses were carried out after alkaline hydrolysis according to Roblin[32]. The calculations are made assuming 3,500 nucleotides in the intact R17 RNA molecule. The percentage of chains with pppGp reflects the fraction of chains of that size which could have 5′ terminal pppGp.

Ribosome Binding to the Fragments

Ribosomes from *E. coli* recognize and shield from nuclease digestion the initiation regions at the beginning of the three cistrons in the R17 RNA[22]. The nucleotide sequences of these sites have been elucidated and the correspondence of each to one of the known R17 proteins has been established.

The distribution of the initiation regions between the 40 per cent and 60 per cent R17 RNA fragments was analysed using the procedure described previously for the whole RNA[22]: MRE600 ribosomes were bound to the radioactive fragments in the presence of formylated charged transfer RNA, initiation factors, and GTP at 5 mM Mg^{2+} concentration (see legend to Fig. 2). After incubation, the ends of the messenger not protected in the initiation complex were trimmed with pancreatic ribonuclease and the digested complex was isolated by sucrose gradient sedimentation. Ribosome binding sites were detected by fingerprint analysis of the radioactive RNA recovered from the 70S region of the gradient.

One set of T_1 ribonuclease fingerprints of initiation sites from the 40 per cent and 60 per cent fragments is shown in Fig. 2. Although the amount of radioactive RNA recovered was insufficient to allow accurate measurement of the relative amounts of the binding sites present in the two fragments, the visual examination of the fingerprints obtained from two sets of fragment preparations reveals that: (1) Oligonucleotides derived from the synthetase binding site (marked S in Fig. 2) are present in higher yield in the 60 per cent than in the 40 per cent fragment (the approximate ratio of 60 per cent to 40 per cent yields is of 3 or 4 : 1). (2) Oligonucleotides specific for the A protein binding site (marked A) are detected in the 40 per cent fragment only, and there at a very low level. (3) Oligonucleotides arising from the coat protein initiation region (marked C) appear primarily in the fingerprint of the binding reaction performed with the 40 per cent fragment (approximate ratio of 3 : 1 or 4 : 1, 40 : 60 per cent).

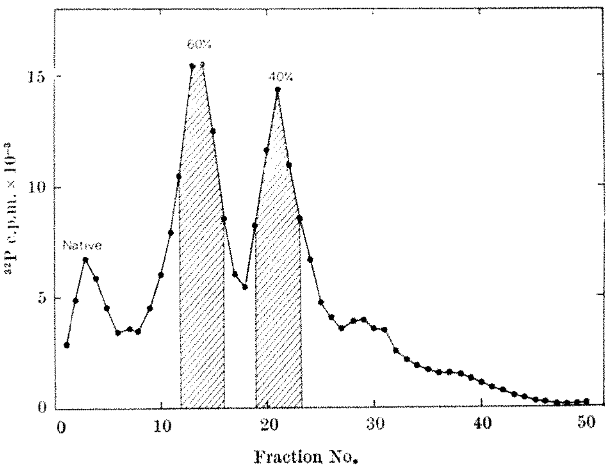


Fig. 1. Sucrose gradient preparation of fragments. ^{32}P-labelled RNA (10^6 c.p.m./μg) was digested with ribonuclease IV as described previously[25] and sedimented for 19 h in a 'Spinco SW 27' rotor at 4° C through a 5–20 per cent sucrose gradient in 0·1 M NaCl, 0·01 M sodium acetate, pH 5·0. Fractions of 0·75 ml. were collected and 1 μl. samples were counted directly in a liquid scintillation counter. Fractions 12–16 and 19–23 (cross-hatched) were pooled to give, respectively, the 60 per cent and 40 per cent preparations used in the experiments described in Figs. 3, 5 and 6 and Tables 1, 3 and 4. R17 phage was grown on ribonuclease I_{10} in low phosphate medium as described previously[9] except that half the normal amount of casamino acids was used. 20 mCi of ^{32}P-orthophosphate was added to 400 ml. of culture 12 min before phage infection. The phage were purified using a new procedure that will be described in detail elsewhere (R. F. Gesteland and P. F. Spahr, in preparation). Briefly, the phages were concentrated by passage of the clarified lysate through a 20 ml. bed volume column of DEAE-cellulose. After washing the column with SSC (0·15 M NaCl, 0·015 M Na citrate, pH 7·0), the phages were eluted with 4 × SSC and banded to equilibrium in CsCl. The phages were freed of small RNA fragments and CsCl by filtration through 'Sephadex G-150'. The RNA extracted from R17 purified in this manner was intact as demonstrated by sedimentation analysis after formaldehyde treatment.

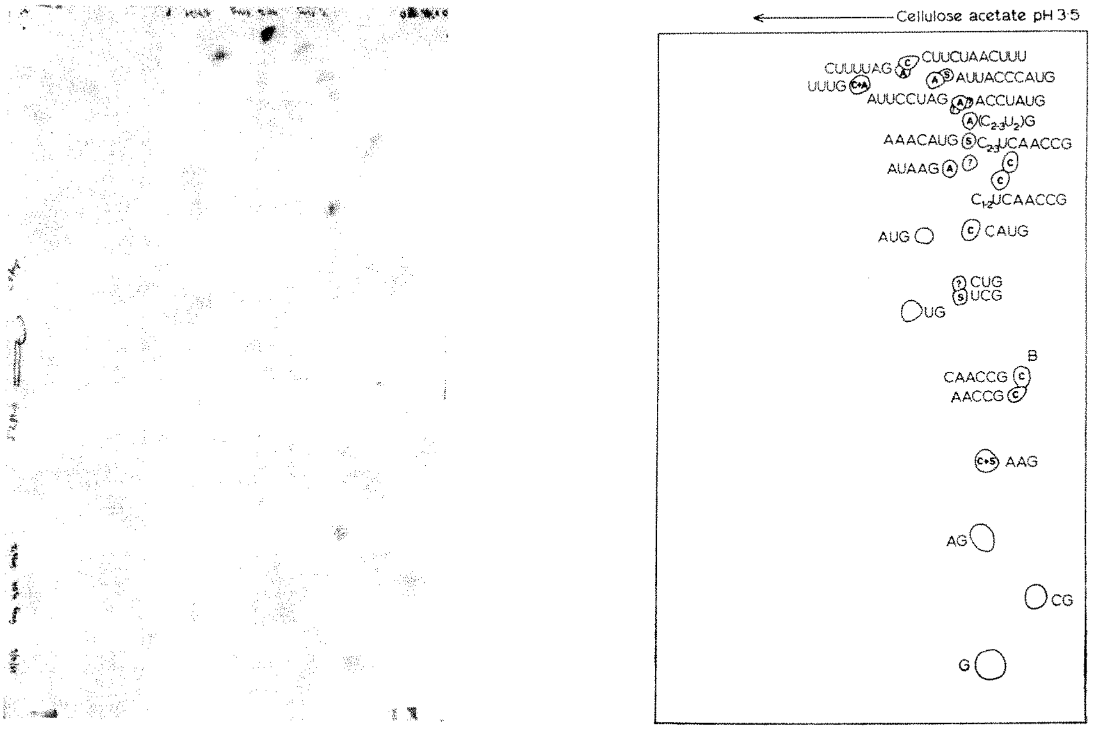
Allocation of T_1 Oligonucleotides to the Fragments

If ^{32}P-labelled R17 RNA is exhaustively digested with T_1 ribonuclease, it is possible to fractionate many of the larger oligonucleotides by a two dimensional fingerprinting system using ionophoresis followed by homochromatography on thin layers of DEAE-cellulose[28]. The characterization of one such T_1 oligonucleotide coding for amino-acids 57 to 61 of the R17 coat protein[5,6] has been described[23].

To isolate further large T_1 oligonucleotides whose sequences could be related to known positions in the R17 RNA molecule, it was necessary to obtain greater resolution in the homochromatography system. This was achieved by partially degrading the RNA of the homomixture, and chromatographing on longer thin layer plates of DEAE-cellulose, as described in the legend to Fig. 3. An autoradiograph of the separation obtained for the T_1 digestion products of the whole R17 RNA molecule is shown in Fig. 3*a*. In addition to the large oligonucleotides many smaller products are resolved if their mobility in the first dimension is particularly high or low.

When the sequences of the well separated spots from such a homochromatogram were investigated, certain of them were found to come from recognizable regions of the R17 genome. Eight of these T_1 oligonucleotides are indicated in Fig. 3*a* and their sequences and origins

T$_1$ RNase fingerprint of initiation sites from 40 per cent fragment



T$_1$ RNase fingerprint of initiation sites from 60 per cent fragment

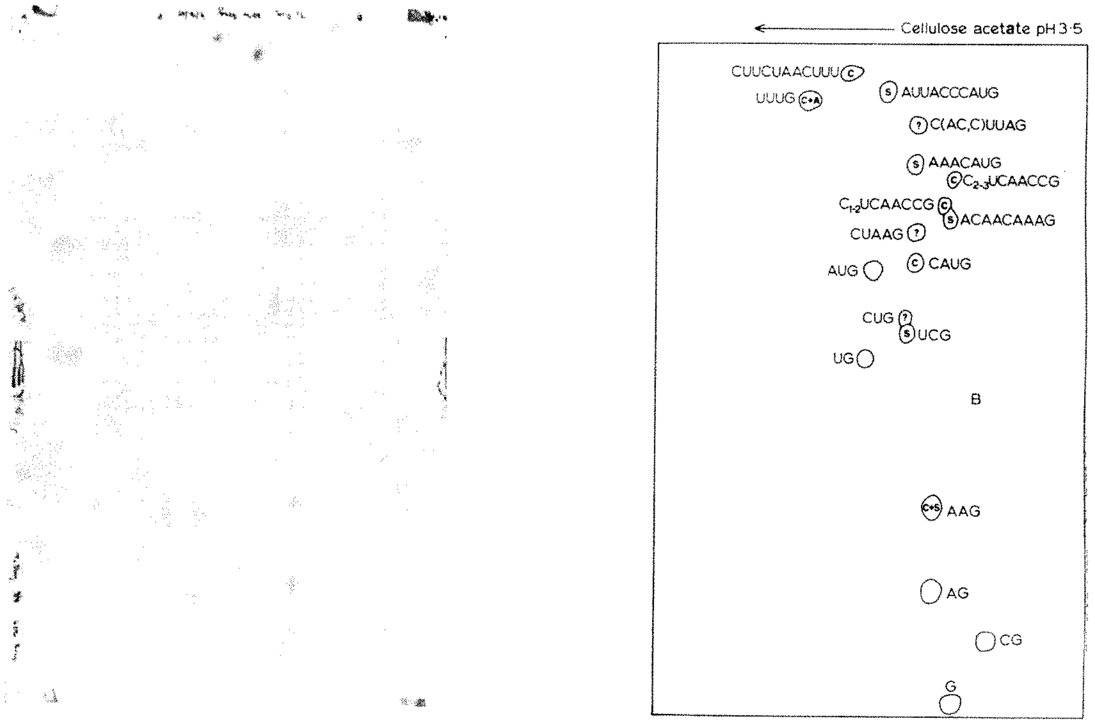
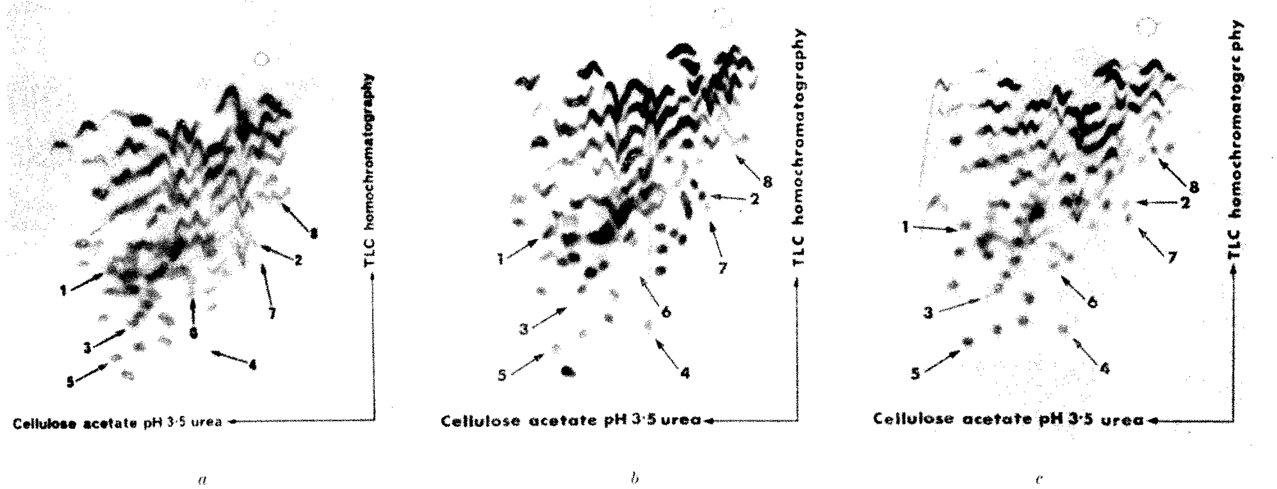


Fig. 2. T$_1$ ribonuclease fingerprints of ribosomal binding sites from the 40 per cent and 60 per cent fragments. Parallel binding reactions were performed at 37° C in 30 μl. of solution containing: 0·1 M Tris chloride (pH 7·4), 0·05 M NH_4Cl, 0·005 M magnesium acetate, 4·0 $A_{260\ nm}$ units of MRE600 ribosomes, 60 μg crude initiation factors, 0·0002 M GTP, 2·8 $A_{260\ nm}$ units of formylated charged mixed tRNA, and either 16 μg ($1·1 \times 10^7$ c.p.m.) of the 60 per cent fragment or 11 μg ($7·7 \times 10^6$ c.p.m.) of the 40 per cent fragment (incubated in 5 μl. of 0·001 M EDTA for 7 min at 37° C before addition). Ribosomes, initiation factors and fMet-tRNA were prepared as described previously[22]. After 12 min of incubation, the binding reaction mixtures were cooled; 0·15 μg of pancreatic ribonuclease was added and incubation at 22° C was continued for 15 min. Following sucrose gradient fractionation[22] the 70S regions were pooled and extracted with phenol in the presence of 0·1 per cent sodium dodecyl sulphate. The resulting 150 μg of RNA was digested with 7 μg of T$_1$ ribonuclease and fingerprinted as described elsewhere[22]. The identity of each oligonucleotide was checked by analysis of its pancreatic ribonuclease digestion products. With the exception of a few spots of unknown origin, each oligonucleotide in the fingerprints is seen to derive from one or another of the three established ribosome binding regions (Fig. 7) as indicated: A = A protein, C = coat protein, S = synthetase. Several oligonucleotides marked A do not appear in the established sequences (Fig. 7); they seem to originate from more extended ribosome-bound fragments and have been identified as linked to the A protein initiation site.



a *b* *c*

Fig. 3. Two-dimensional thin layer homochromatography fingerprints of T_1 digests of intact R17 RNA (*a*), the 40 per cent fragment (*b*) and the 60 per cent fragment (*c*). For the intact molecule, 20 μg of ^{32}P-RNA (about 10^7 c.p.m.) was digested with 1 μg of ribonuclease T_1 in 3–4 μl. of 0·01 M Tris-HCl, *p*H 7·4, containing 0·001 M EDTA, at 37° C for 30 min. The products were fractionated by ionophoresis on cellulose acetate in 7 M urea, *p*H 3·5, as a first dimension, followed by homochromatography[28], using homomixture *c* with DEAE-cellulose and cellulose in the ratio 1 : 7·5 on a 20 cm × 40 cm plate. For the fragments about 10^6 c.p.m. of each was made up to 20 μg with non-radioactive carrier tRNA: T_1 digestion and fractionation of products were performed as above. The numbered spots are identified in Table 2.

listed in Table 2. Sequence *1* is obtained by J. M. Adams and S. Cory (personal communication) in an R17 RNA fragment of approximately 75 nucleotides containing pppGp, the 5′ terminus of the RNA molecule. As shown in Fig. 4, sequences *3*, *4* and *5* are related to amino-acid sequences in the coat protein[5,6] *3* being an extension of the coat protein ribosome binding site[22] (Fig. 7). (Sequence *3* is the same as that proposed by Robinson, Frist and Kaesberg[29] from work with a strain of R17 having an amber mutation at position 6 of the coat protein.) Sequence *6* is homologous with the beginning of the synthetase binding site[22] (Fig. 7) and is found in an RNA fragment containing, and extending beyond, the 3′-terminus of the coat protein cistron[24]. Sequence *8* is the 3′-end of the R17 RNA molecule[30]. Owing to their size and composition, it was not possible to locate any of the A protein binding site T_1 oligonucleotides as pure spots on this homochromatographic system.

Figs. 3*b* and 3*c* are fingerprints of the 40 per cent and 60 per cent fragments, analogous to that of the whole molecule (Fig. 3*a*). With the exception of oligonucleotide *3* which appears in low yield in both fragments (the significance of which will be discussed later), each of the well resolved oligonucleotides occurs in major yield in one or other of the fragment preparations, but not in both. This finding supports the idea of a single primary site of cleavage by ribonuclease IV in the R17 RNA molecule, giving rise to the two fragments. The presence of sequence *1* as a major spot in the 40 per cent fingerprint, and similarly of sequence *8* in the 60 per cent fingerprint, confirms the fragment orientation in the original RNA molecule based on pppGp analyses[16,25,26].

Table 2. SEQUENCES AND LOCATION OF SPECIFIC T_1 OLIGONUCLEOTIDES

Oligonucleotide (see Fig. 3)	Sequence	Location
1	CCAUUUUUAAUG	Approximately 50 residues from the 5′-end
2	(C)CCUCAACCG*	Coat protein ribosome binding site, proximal to initiating AUG
3	CUUCUAACUUUACUCAG	Coat protein cistron (amino-acids 1–6)
4	CAAAUACACCAUUAAAG	Coat protein cistron (amino-acids 57–61)
5	AAUUAACUAUUCCAAUUUUCG	Coat protein cistron (amino-acids 89–95)
6	CCAUUCAAACAUG	Synthetase ribosome binding site
7	ACAACAAAG	Synthetase ribosome binding site
8	$\text{UUACCACCCA}_{\text{OH}}$	3′-end

* It is not clear whether this oligonucleotide has two or three C residues at the 5′-end, although the best data at present suggest there are three.

The methods used to verify each of the above sequences will be published elsewhere (P. G. N. J., unpublished results).

3 (G)CU UCU AAC UUU ACU CAG
f-Met Ala Ser Asn Phe Thr Gln . . .
1 5

4 (G)C AAA UAC ACC AUU AAA G
. . . Arg Lys Tyr Thr Ile Lys Val . . .
57 60

5 (G)AA UUA ACU AUU CCA AUU UUC G
. . . Glu Leu Thr Ile Pro Ile Phe Ala . . .
90 95

Fig. 4. Sequences of T_1 oligonucleotides *3*, *4* and *5*, showing their relationship to amino-acid sequences in the R17 coat protein[5,6].

A quantitative measure of the yields of oligonucleotides *1*–*8* in the 40 per cent and 60 per cent fragments was obtained by scraping the appropriate spots off the two fingerprints and counting them directly (Table 3, columns (*a*) and (*c*). The identity of each was then checked by analysis of its pancreatic ribonuclease digestion products. Sequences *1* and *8* can conveniently be used as standards with which to compare the yields of the other oligonucleotides as tabulated in columns (*e*) and (*f*) of Table 3. Sequence *2* is present in the 40 per cent fragment in 87 per cent yield whereas *3* to *8* appear in 25 per cent yield or less. Conversely, the 60 per cent fragment contains oligonucleotides *4* to *7* in better than 80 per cent yield and *1* to *3* in less than 23 per cent yield. The relative recovery of some oligonucleotides may be low, for the efficiency of transfer from the first dimension to the DEAE-cellulose thin layer[28] may vary both along the length of the cellulose acetate strip and with the size and/or composition of the oligonucleotide. Nevertheless,

Table 3. YIELDS OF SPECIFIC T_1 OLIGONUCLEOTIDES FROM THE 40 PER CENT AND 60 PER CENT FRAGMENTS

Oligonucleotide (No. of phosphates)	(*a*) 40 per cent C.p.m. (total)	(*b*) 40 per cent C.p.m. per phosphate	(*c*) 60 per cent C.p.m. (total)	(*d*) 60 per cent C.p.m. per phosphate	(*e*) Percentage yield (see text) 40 per cent relative to *1*	(*f*) Percentage yield (see text) 60 per cent relative to *8*
1 (12)	575	47·9	76	6·3	—	23
2 (10*)	416	41·6	57	5·7	87	21
3 (17)	86	5·1	59	3·5	11	13
4 (17)	146	8·6	376	22·1	18	80
5 (21)	232	11·0	538	25·6	23	92
6 (13)	130	10·0	286	22·0	21	80
7 (9)	107	11·9	249	27·7	25	100
8 (9)	78	8·7	249	27·7	18	—

* See footnote to Table 2.

Glass sinters were counted before and after taking up the DEAE-cellulose corresponding to the marker T_1 oligonucleotides in a 'Unilux I' (Nuclear Chicago) scintillation counter, without the addition of scintillator (setting C50). The differences between these two readings are tabulated in columns (*a*) and (*c*).

After its radioactivity had been counted, each oligonucleotide was eluted and its identity checked by analysis of its pancreatic ribonuclease digestion products. The radioactivity from spots observed in minor yield was shown to come from the oligonucleotides in question rather than from contaminants.

the data allow unequivocal assignment of oligonucleotides *2* to *7* (with the exception of *3*) either to the 40 per cent or to the 60 per cent fragment. The level of contamination of each fragment by the other is consistent with the pppGp analyses and the results of ribosome binding.

Table 4. RELATIVE YIELDS OF A PROTEIN INITIATION SITE OLIGONUCLEOTIDES FROM COMPLEXES FORMED BETWEEN *B. stearothermophilus* RIBOSOMES AND THE 40 PER CENT AND 60 PER CENT FRAGMENTS

Oligonucleotide	Counts per min 40 per cent	Counts per min 60 per cent	Relative yield 40 per cent : 60 per cent
UUUG	6,450	1,200	5·4
CUUUUAG	7,350	1,500	4·9
ACCUAUG	10,400	1,850	5·6

Oligonucleotide spots were cut from the fingerprints shown in Fig. 6 and counted in a toluene scintillator. The actual counts per min obtained for the 60 per cent oligonucleotides have been multiplied by a factor of 1·75 to adjust for the fact that non-equimolar amounts of the two fragments were used in the initial binding reactions. The 40 per cent : 60 per cent ratio for CUUUUAG is probably least accurate because this oligonucleotide appears near the 3′-end of the A protein ribosomal binding site (see Fig. 7) and therefore often experiences internal cleavage upon pancreatic ribonuclease digestion of the initiation complex.

Location of A Protein Initiation Site

Because no unique T_1 oligonucleotide from the A protein initiation site could be identified in the homochromatogram of a complete T_1 ribonuclease digest of R17 RNA, an alternative method was used to estimate the amounts of A protein initiation site in the 40 per cent and 60 per cent fragments. When ribosomes from *Bacillus stearothermophilus*, rather than from *E. coli*, are used for the *in vitro* translation of f2 RNA, only the A protein is initiated and synthesized[31]. Similarly with R17 RNA[22], the A protein initiation site alone binds to *B. stearothermophilus* ribosomes; no recognition of the coat protein or synthetase sites can be detected. Moreover, at 65° C, *B. stearothermophilus* ribosomes attach to the beginning of the A protein cistron with extremely high efficiency. At least 50 per cent of the theoretical number of A sites can be recovered from *B. stearothermophilus* ribosomes as compared with approximately 3 per cent from *E. coli* ribosomes (J. A. S., unpublished observation).

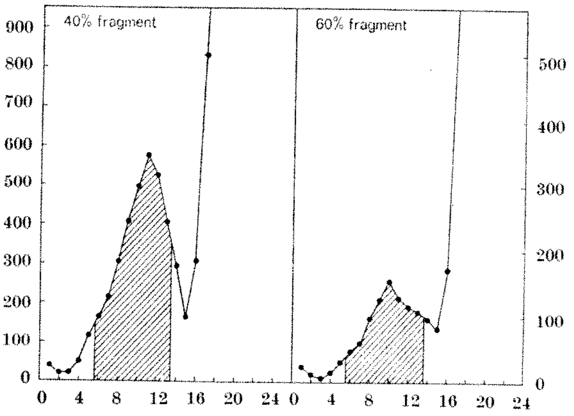


Fig. 5. Initiation complexes formed between ribosomes from *B. stearothermophilus* and the 40 per cent and 60 per cent fragments. Parallel binding reactions were performed at 66° C in 40 μl. of solution containing: 0·1 M NH_4 cacodylate (*p*H 7·1), 0·1 M NH_4Cl, 0·008 M magnesium acetate, 0·0002 M GTP, 4·5 $A_{260\text{ nm}}$ units of *B. stearothermophilus* ribosomes (prepared as described previously[22]), 4·0 $A_{260\text{ nm}}$ units of formylated charged mixed tRNA, and either 42 μg ($1·4 \times 10^7$ c.p.m.) of the 40 per cent fragment or 36 μg ($1·2 \times 10^7$ c.p.m.) of the 60 per cent fragment. After 8 min of incubation, the binding reaction mixtures were cooled; 0·2 μg of pancreatic ribonuclease was added and incubation continued for 15 min at 22° C. Sucrose gradient centrifugation was performed as described previously[22] and 1/40 volume aliquots of the fractions were counted. In the diagram the ^{32}P c.p.m. scales have been adjusted because non-equimolar amounts of the two fragments were used in the binding reactions. The fraction of A protein sites bound was calculated assuming that the average length of an initiator fragment is 30 nucleotides. Because 1 per cent of the radioactivity initially present in the 40 per cent fragment binding reaction and 0·3 per cent of that in the 60 per cent reaction appear in the 70S region of the gradients, a total of about 60 per cent of the theoretical number of A sites was recovered.

Portions of the same 40 per cent and 60 per cent fragment preparations used for pppGp analyses and homochromatography (Fig. 3 and Tables 1 and 3) were incubated with *B. stearothermophilus* ribosomes, treated with nuclease and sedimented through sucrose gradients as shown in Fig. 5. It is clear that significantly more radioactive RNA is protected when the initiation complex is made with the 40 per cent fragment than with the 60 per cent fragment. Furthermore, fingerprint analyses (Fig. 6) of the material indicated as cross-hatched in Fig. 5 demonstrate that virtually all the ribosome bound radioactive RNA from the 40 per cent fragment derives from the A protein initiation site (see Fig. 7). The material from the 60 per cent fragment gives a less clean fingerprint, containing a higher background of non-specific oligonucleotides. The three large T_1 oligonucleotides arising from the interior of the A protein binding site were cut from the two fingerprints and counted directly (Table 4). In this experiment, in which approximately 60 per cent of the theoretical number of A sites were recovered, a five-fold higher yield of the A protein initiation region from the 40 per cent than from the 60 per cent fragment is observed.

Biochemical Map for R17 RNA

From the data given in Tables 3 and 4, it is possible to construct the biochemical map of the R17 genome illustrated in Fig. 7. The crucial assignment of the coat protein gene to the middle cistron is unambiguous, for T_1 oligonucleotide *2*, which arises from the 5′ end of the coat protein ribosomal binding site, is found in good yield in the 40 per cent fragment, whereas oligonucleotides *4* and *5*, from the interior of the coat protein gene, are recovered primarily from the 60 per cent fragment. Thus ribonuclease IV most often cleaves the R17 RNA somewhere between the beginning of the coat gene and the nucleotides coding for amino-acids 57 to 61 of this protein. The low yield of oligonucleotide *3* from both the 40 per cent and the 60 per cent fragment preparations further pinpoints the principal site of ribonuclease IV attack to the region coding for the first six amino-acids of the coat protein and locates this oligonucleotide at a position about 40 per cent of the way along the R17 RNA molecule.

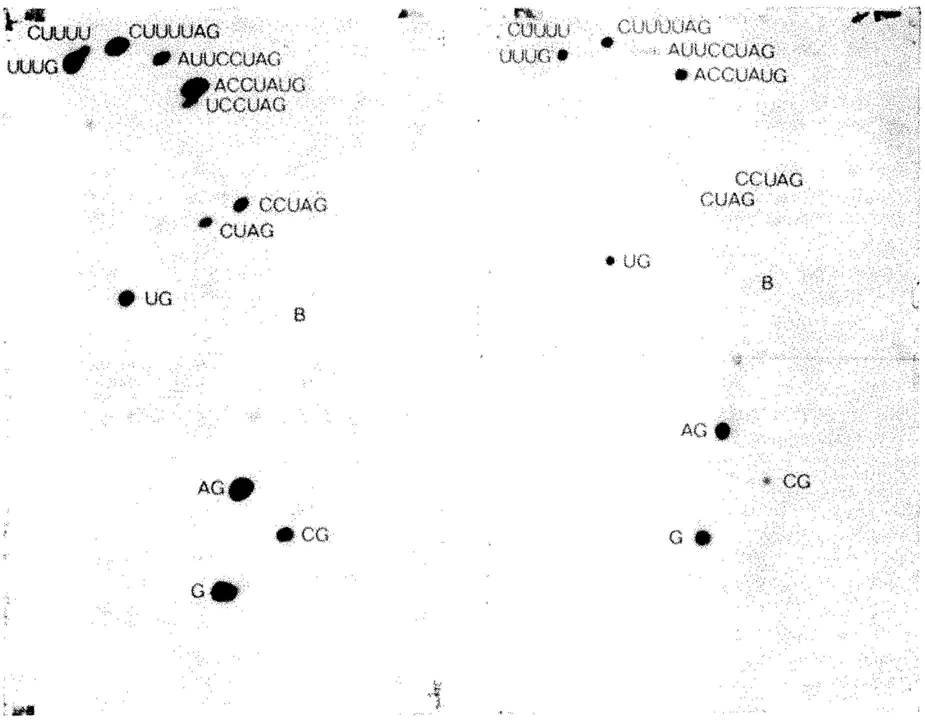


Fig. 6. T_1 ribonuclease fingerprints of initiation complexes formed between *B. stearothermophilus* ribosomes and the 40 per cent and 60 per cent fragments. Left: 40 per cent; right: 60 per cent. Fractions 6–13 from the sucrose gradients illustrated in Fig. 5 were pooled and extracted with phenol in the presence of 0·1 per cent sodium dodecyl sulphate. T_1 ribonuclease digestion and fingerprint analyses of the resulting RNA were performed as described previously[22]. Because non-equimolar amounts of the two fragments were used in the initial binding reactions, the 40 per cent fingerprint was exposed for 30 h, and the 60 per cent for 52 h to allow direct comparison.

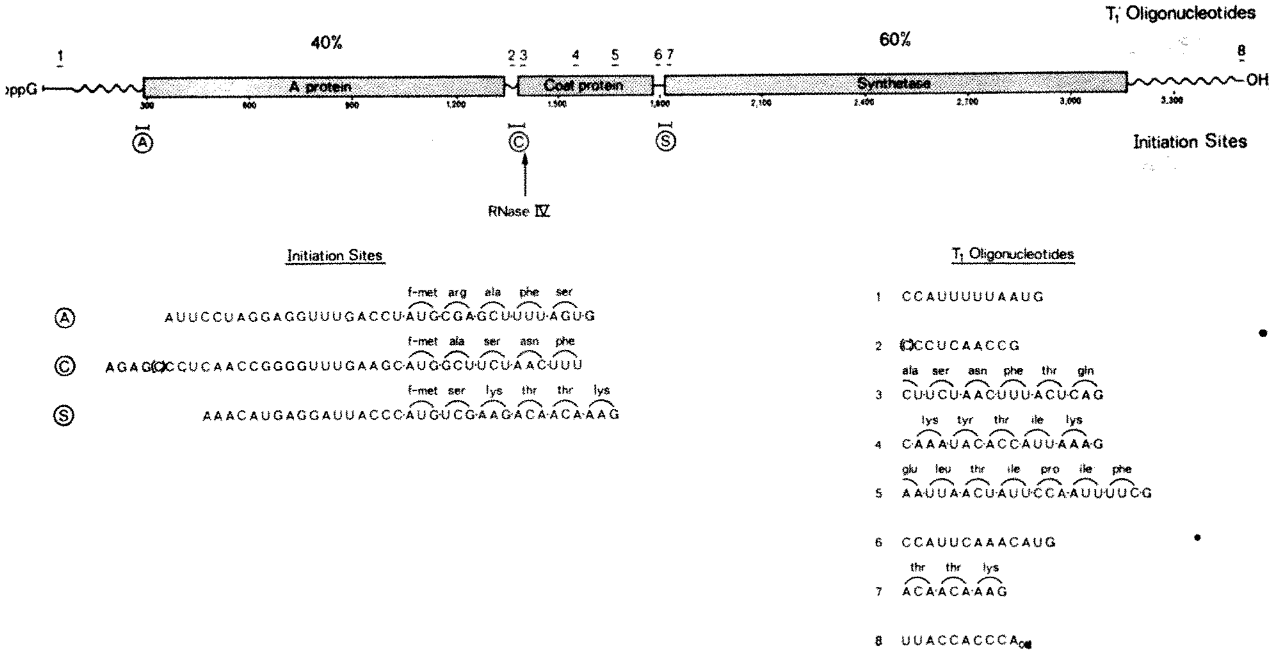


Fig. 7. Biochemical map of the R17 RNA (drawn to scale). An RNA chain length of 3,500 nucleotides has been assumed and the best molecular weight estimates for the A protein and synthetase were used. The eight specific T_1 oligonucleotides and the ribosomal binding sites have been located as accurately as possible along the genome. Wavy lines indicate that the actual lengths of the corresponding regions are unknown.

Once the position of the coat protein cistron has been assigned, it follows that each of the remaining genes must be wholly contained in one or the other of the two R17 RNA fragments. The high yields in the 60 per cent fragment of oligonucleotides *6* and *7*, which arise from the initiation region of the synthetase cistron, place this gene at the 3′ end of the RNA; and the results of the binding experiments performed with *B. stearothermophilus* ribosomes strongly support the location of the A cistron nearest the 5′ terminus.

The recent elucidation of the nucleotide sequence of the entire intercistronic region between the end of the coat protein and the beginning of the synthetase genes[22,24] eliminates any doubt that the synthetase cistron directly follows the coat protein cistron in the R17 genome. Our knowledge of the sequence also allows us to draw this region to scale (36 nucleotides) in the biochemical map. The size of the intercistronic region between the A protein and the coat protein cistrons has been assumed to be the same.

The untranslated regions at the two ends of the R17 genome are the major uncertainties remaining in the map. The first 75 nucleotides (and probably the first 100 nucleotides) do not overlap the A protein initiation site (J. M. Adams and S. Cory, personal communication) and at least 11 nucleotides at the 3′ end of the RNA are not included in the synthetase cistron[30]. But because the RNA has been variously estimated to contain 3,300[9,10] to 3,500[11] nucleotides and the molecular weights of the A and synthetase proteins have not been precisely determined, both the total lengths and the manner of distribution of the remaining untranslated material between the termini of the R17 RNA molecule are unknown.

Ribonuclease Cleavage and RNA Conformation

Identification of the principal site of ribonuclease IV attack in the R17 RNA molecule clarifies many results previously obtained in studies of the 40 per cent and 60 per cent fragments[16,25]. Although the complete coat protein ribosomal binding site is usually retained by the 40 per cent fragment (preliminary results of J. M. Adams and S. Cory (personal communication) suggest that ribonuclease IV cleavage may occur specifically after the phenylalanine codon UUU), any polypeptides produced would be too short to be detected in the conventional assay systems. This, in addition to the low level of A protein synthesis in extracts from *E. coli*[14,15], explains the relative inactivity of the 40 per cent fragment as a messenger *in vitro*[16,25]. The 60 per cent fragment, on the other hand, efficiently directs synthetase production; the amount of coat protein it initiates (20 per cent to 25 per cent that of the intact RNA[16,25]) might suggest a second site of attack by ribonuclease N (ref. 25), but the degree of purity of the fragment preparations examined here is not sufficient to reveal more than the one major site of attack.

The fact that ribonuclease IV cleaves in the vicinity of the coat protein initiator strengthens previous suggestions that this area lies in an exposed region of the intact R17 RNA molecule[25]. Because several nucleases in addition to ribonuclease IV yield fragments of about 22S and 15S as the first cleavage products (refs. 33–35 and personal communication of H. Boedtker and H. D. Robertson), it seems that isolated bacteriophage RNAs may possess not only ordered regions of secondary structure[22–24,27] but also a well defined tertiary structure. The beginning of the coat gene may be most readily accessible not only to nuclease attack but also to ribosome attachment, possibly contributing to the high relative frequency of initiation of coat protein synthesis.

Translation and Gene Order

The genome of the RNA bacteriophages, either *in vivo* or *in vitro*, directs the synthesis of very different amounts of the three proteins. For example, the molar amount of coat protein made can exceed that of synthetase and A protein by three and twenty-fold respectively[14]. Translation is also subject to a polar effect whereby an amber mutation at position 6 of the coat protein drastically lowers the production of synthetase[36–39,42]. Because both these phenomena are demonstrable *in vitro*, they must reflect intrinsic properties of the phage RNA molecule, and any explanation of them must be consistent with the gene order.

Polarity and the high relative rate of coat protein synthesis have suggested that the RNA bacteriophage genome is read sequentially[38,40], starting with the coat cistron. The gene order established here, however, proves that translation of the R17 RNA cannot be wholly sequential. Moreover, because the A protein (which is made in smallest amount), is encoded before the coat protein, independent entry of ribosomes at the beginning of the coat gene is obligatory. The actual levels of synthesis of the three proteins very likely reflect different rates of initiation, which in turn may be influenced not only by the nucleotide sequences of the initiation regions but also by the secondary and tertiary structure of the RNA molecule.

Because ribosomes have independent access to initiation sites on fragmented RNA[14,22,41], polarity also cannot depend on sequential reading of the phage genome. In other words, the fact that the synthetase gene follows the coat cistron may be irrelevant; polarity could arise from defined secondary or tertiary structure in the intact phage RNA molecule[14,22,25,36,37,39]. The initially inaccessible synthetase binding site could be made accessible by changes in the folding of the RNA brought about either by the ribosome as it translates the coat cistron or by the removal of large segments of the molecule through nuclease action (suggested by the fact that position 6 amber mutants in the 60 per cent fragment seem to have lost their polarity[25]).

In conclusion we stress that the RNA bacteriophage genome may not be the typical operon that is so frequently assumed. The messenger activity appears to be subject to strict secondary and tertiary structure constraints, imposed possibly because it must fold efficiently into a spherical virus particle. Whether similar mechanisms operate in bacterial and other messengers is not known.

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Comparison of the Partial Amino-acid Sequence of Dogfish Trypsinogen with Bovine Trypsinogen

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Trypsinogen molecules from two species of different evolutionary periods are compared. Homology has been established by the sequencing of over half the amino-acids of the dogfish enzyme.

THE concept of homology in primary structure has now been amply documented in a variety of protein and enzyme systems[1,2]. In particular, it has been shown that a significant degree of homology exists within the class of mammalian serine proteases, including trypsin, chymotrypsins A and B, elastase and thrombin[3]. Recent investigations have extended these results to show that the three-dimensional structure also reflects these similarities[4]. It may be tentatively concluded that enzymes with homologous amino-acid sequences possess similar three-dimensional structures as well. In order to extend the analysis for homology to species of earlier evolutionary periods, we have examined the chemical structure of trypsinogen isolated from the pancreas of the spiny Pacific dogfish with the view of making a detailed comparison with the analogous bovine zymogen. A preliminary examination of the primary structure has now been completed and certain points of similarity can already be discerned.

Dogfish trypsinogen was isolated from freshly excised pancreas glands of dogfish caught in and around the waters of Puget Sound. After extraction and chromatography, the crude trypsinogen fraction was adjusted to *p*H 3, treated with ammonium sulphate, and finally purified on 'SE-Sephadex' (personal communication from R. Haynes, W. P. W. and R. W. T.).

Amino-acid Composition and Structure

The amino-acid composition of dogfish trypsinogen was determined from acid hydrolysates as listed in Table 1 and compared with the composition of the bovine zymogen. Differences in several amino-acids, such as serine, methionine, arginine, histidine and lysine, are evident. Yet, when compared with five other trypsinogens or trypsins, including bovine, porcine[8], ovine[9], human[10] and starfish (H. N. and W. P. W., manuscript in preparation), only the methionine and histidine contents of the dogfish protein seem at variance to the averages, though the serine and threonine total is somewhat lower than the others[11].

The determination of the amino-acid sequence was initiated by isolation of the tryptic peptides from trypsinogen reduced and alkylated with ethylenimine. All of the tryptic peptides were soluble. Digests of S-(β-aminoethyl)-trypsinogen and native trypsinogen with pepsin were also prepared. Tryptic and peptic digests of S-(β-aminoethyl)-trypsinogen were separated on 'Dowex 50 × 8' with double linear gradients of pyridine acetate[12]. The fractions were tested for purity by electrophoresis and subfractionated on either 'Dowex 1 × 2' or 'Dowex 50 × 2'[14]. Purified peptides were sequenced by Edman degradation and by

Table 1. THE AMINO-ACID COMPOSITION OF DOGFISH TRYPSINOGEN I

Amino-acid	Dogfish*	Bovine[7]
Lysine	6·3	15
Histidine	7·6	3
Arginine	6·5	2
Aspartic acid	27·9	26
Threonine	7·3†	10
Serine	16·5†	33
Glutamic acid	15·5	14
Proline	11·3	9
Glycine	27·8	25
Alanine	17·0	14
Half-cystine	11·9‡	12
Valine	17·4§	18
Methionine	8·1‡	2
Isoleucine	13·5§	15
Leucine	14·6	14
Tyrosine	11·5	10
Phenylalanine	1·2	3
Tryptophan	5·4¶	4
Total	227·3	229

* Average of five determinations, each at 18, 24, 36, 48 and 96 h of hydrolysis.
† Extrapolated to zero time.
‡ Determined after performic acid oxidation[5].
§ Values for 96 h hydrolysis.
¶ Determined spectrophotometrically by the method of Edelhoch[6].

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carboxypeptidase A and B hydrolysis[13]. The peptic peptides from native trypsinogen were purified on columns of 'SE-Sephadex' (R. A. B. and K. A. W., submitted for publication) and subfractionated on columns of Dowex as before. Of the three digests, only the tryptic peptides were extensively examined.

The composite structure, deduced from the peptides isolated from these three digests, is shown in Fig. 1. In view of the preliminary nature of the data, it is not surprising that many of the requisite overlaps needed to produce a rigorous structure are missing, although it was readily apparent early in these studies that a high degree of homology to the bovine protein did exist and that the known structure of the bovine protein could therefore serve as a tentative guide for aligning the various peptides isolated from the dogfish zymogen. Thus the structure presented in Fig. 1 relies heavily on the bovine structure as a template, and any conclusions regarding their homologous relation must be viewed within that limitation at the present time.

Comparison with the Bovine Enzyme

As shown in Fig. 1, 205 of the 229 amino-acids comprising the bovine protein are accounted for by peptides isolated from one or more of the three digests of the dogfish protein. Of these 205, 123 have been positioned by sequence analysis and can be compared with the bovine enzyme. Eighty-five (69 per cent) were found to possess the same amino-acid residue in both proteins. Thus even in the absence of the complete sequence, the similarity of these two proteins is sufficient to be certain of their homology.

The functional residues that have been identified from chemical modification experiments[14,15] and by deduction from X-ray data of the structurally homologous enzyme α-chymotrypsin[16] can all be located in the portions of the molecule presented. Histidine 46, serine 183, and possibly aspartic acid 90 (ref. 3)—all catalytically significant residues—and aspartic acid 177, which has been implicated as the binding site of the positive charge associated with the side chain of the tryptic substrates[17], have been placed directly or by compositional data. In addition, the sequences surrounding these residues are almost identical.

The analysis also revealed evidence for amino-acid replacement sites. In one case, two peptides of closely similar composition and structure apparently correspond to residues 169–176. Whereas the bovine protein possesses alanine, tyrosine and leucine at positions 169, 171 and 172, one peptide of the dogfish protein contains valine, tyrosine and methionine, while the other contains alanine, phenylalanine and leucine, respectively, on the basis of the best fit of the composition. In the second case, the

```
                1                                   10                                      20
Bovine          VAL-ASP-ASP-ASP-ASP-LYS-ILE-VAL-GLY-GLY-TYR-THR-CYS-GLY-ALA-ASN-THR-VAL-PRO-TYR-
Dogfish        [ALA-PRO-ASX-ASX-ASX (ASX, LYS, ILE, VAL, GLY, GLY, TYR, GLX, CYS, PRO, LYS)]

                21                                  30                                      40
Bovine          GLN-VAL-SER-LEU-ASN-SER-GLY-TYR-HIS-PHE-CYS-GLY-GLY-SER-LEU-ILE-ASN-SER-GLU-TRP-
Dogfish                        [ASX-VAL-GLY-TYR-HIS-PHE-CYS (GLY, GLY, SER) LEU] [ILE-HIS-GLX-GLX]

                41                                  50                                      60
Bovine          VAL-VAL-SER-ALA-ALA-HIS-CYS-TYR-LYS-SER-GLY-ILE-GLN-VAL-ARG-LEU-GLY-GLN-ASP-ASN-
Dogfish                    [ALA-(ALA, HIS, CYS, TYR, GLY, SER, ARG)] [ILE-GLN-VAL-ARG] [LEU-GLY-GLX-HIS-ASX-

                61                                  70                                      80
Bovine          ILE-ASN-VAL-VAL-GLU-GLY-ASP-GLU-GLU-PHE-ILE-SER-ALA-SER-LYS-SER-ILE-VAL-HIS-PRO-
Dogfish         ILE-SER-ALA-ASX-(GLX, GLY, ASX, THR, GLX)-TYR-ILE-ASP-SER-SER-MET (VAL, ILE)-ARG] [HIS-PRO-

                81                                  90                                      100
Bovine          SER-TYR-ASN (PRO, LEU, THR, ASN) ASN-ASN-ASP-ILE-MET-LEU-ILE-LYS-LEU-LYS-SER-ALA-ALA-
Dogfish         ASX-TYR (GLY, SER)(LEU, TYR, ASX, ASX)(ASN, ASP) ILE-MET-LEU-ILE-LYS-LEU-SER-LYS-PRO-ALA-

                101                                 110                                     120
Bovine          SER-LEU-ASN-SER-ARG-VAL-ALA-SER-ILE-SER-LEU-PRO-THR-SER-CYS-ALA-SER-ALA-GLY-THR-
Dogfish         ALA-LEU-ASN-ARG-ASP-VAL-ASN-LEU-ILE-SER-LEU-PRO-THR-GLY-CYS-ALA-TYR-ALA-GLY-GLU-

                121                                 130                                     140
Bovine          GLN-CYS-LEU-ILE-SER-GLY-TRP-GLY-ASN-THR-LYS-SER-SER-GLY-THR-SER-TYR-PRO-ASP-VAL-
Dogfish         MET-CYS]-[LEU-ILE-SER-GLY-TRP-GLY-(ASX, THR, ASX, GLY, MET) ALA-VAL-SER-    -GLY-ASX-GLX-

                141                                 150                                     160
Bovine          LEU-LYS-CYS-LEU-LYS-ALA-PRO-ILE-LEU-SER-ASP-SER-SER-CYS-LYS-SER-ALA-TYR-PRO-GLY-
Dogfish         LEU-GLX-CYS-LEU-ASP-ALA-PRO-VAL (ALA, ASP, LEU, SER, GLU) CYS-LYS] [GLY-ALA-TYR-PRO-GLY-

                161                                 170                                     180
Bovine          GLU-ILE-THR-SER-ASN-MET-PHE-CYS-ALA-GLY-TYR-LEU-GLU-GLY-GLY-LYS-ASP-SER-CYS-GLN-
Dogfish         (MET, ILE, THR, ASN, ASN)-MET-MET-CYS] [ALA-GLY-(PHE, LEU, GLX, GLY, GLY) LYS] [ASP-SER-CYS] [(GLX,
                                                       [VAL-GLY-TYR-MET-GLX-GLY-GLY-LYS]

                181                                 190                                     200
Bovine          GLY-ASP-SER-GLY-GLY-PRO-VAL-VAL-CYS-SER-GLY-LYS-LEU-GLN-GLY-ILE-VAL-SER-TRP-GLY-
Dogfish         GLY, ASX, SER, GLY, GLY, PRO, VAL)-VAL-CYS]              [(GLX, GLY, ILE, VAL, SER)]

                201                                 210                                     220
Bovine          SER-GLY-CYS-ALA-GLU-LYS-ASN-LYS-PRO-GLY-VAL-TYR-THR-LYS-VAL-CYS-ASN-TYR-VAL-SER-
Dogfish                    [(ALA, GLX, ARG)] [ASX-HIS-PRO-GLY-VAL-TYR-THR-ARG]

                221                             229
Bovine          TRP-ILE-LYS-GLN-THR-ILE-ALA-SER-ASN
Dogfish            [ILE-HIS-GLX-THR-ILE-ALA-SER-ALA/VAL]
```

Fig. 1. Comparison of the amino-acid sequence of bovine trypsinogen[7] with the partial sequence of dogfish trypsinogen. Peptides positioned by analogy to the bovine protein are indicated by brackets. Unknown sequences are indicated by parentheses. Except at position 137, blank areas indicate lack of peptides to complete these positions at present.

carboxyl terminal residue of the protein is occupied by either alanine or valine.

Several other points of interest deserve comment. First, the amino terminal portion of the molecule, which has been partly sequenced, indicates that dogfish trypsinogen probably contains an activation peptide with the sequence H-Ala–Pro–Asx–Asx–Asx–Asx–Lys. The position of the sixth and seventh residues has not been confirmed nor has the nature of the side chain groups of the four aspartic acid/asparagine residues. By analogy with other trypsinogens[9], these are likely to be aspartic acid. It is of interest to note that tryptic digestion of S-(β-aminoethyl)-trypsinogen did not cleave the lysyl–isoleucine bond associated with activation of the native zymogen, an observation in accord with that of Pèchere *et al.*[18] with bovine S-sulphotrypsinogen.

Second, the tyrosyl residue at position 137 of the bovine protein has apparently been deleted in the dogfish protein because four different peptides from both the tryptic and peptic digestions have been assigned to this region. Because dogfish trypsinogen has an additional residue at the N-terminus, the two proteins are of similar if not identical length.

Although the cystinyl peptides have not yet been fully characterized, it is likely that the positions of the disulphide bonds in the dogfish protein are identical to those in the bovine protein. In fact, the ten residues of half-cystine already located occur at identical positions in both proteins. Only two residues of half-cystine, at positions 203 and 216, have not been isolated, but because these two residues are bonded in the bovine protein to the half-cystinyl residues at positions 179 and 115, respectively, and are present in identical positions in the dogfish protein, it may be supposed that the missing half-cystinyl residues occupy identical, or very nearly identical, positions in the dogfish protein. The probable identity of the disulphide bridges as well as the high degree of homology already documented suggest that the three-dimensional structures of these two proteins will be very similar.

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Plate Tectonics of the Mediterranean Region

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The seismicity and fault plane solutions in the Mediterranean area show that two small rapidly moving plates exist in the Eastern Mediterranean, and such plates may be a common feature of contracting ocean basins. The results show that the concepts of plate tectonics apply to instantaneous motions across continental plate boundaries.

THE hypothesis of sea floor spreading[1-5] has been extended recently to include all motions observed at the Earth's surface. The additional hypothesis required is that all aseismic areas of the surface move as rigid plates without internal deformation. The new theory is often called "plate tectonics", to distinguish it from earlier and less precise versions of the theory of continental drift. Plate tectonics was first suggested by Wilson[6] and by Bullard *et al.*[7], but has been applied only recently to the tectonics of ridges and trenches[8-11]. It is clear from this recent work that the assumption of plate rigidity is justified, and that plate tectonics provides an excellent description of present motions. In its original form the theory had, however, several limitations. Perhaps the most important was that the evolution of plates was not considered at all; the theory was essentially an instantaneous theory only. This limitation has now been partly overcome[12]. Another major shortcoming was that the simple concepts of plate tectonics could not be applied to continental boundaries. The reason for this difficulty is the complicated nature of most plate boundaries wherever they cross continents. In such regions no sharp seismic boundary exists, and the focal mechanisms of earthquakes do not reflect the motion of the major plates on each side in any simple way (Fig. 1). This behaviour is in striking contrast to the properties of plate boundaries in oceanic areas[11], where the majority of earthquake mechanisms are directly related to the plate movements and the width of the seismic zones is often less than the present errors in epicentre locations. This difference between the tectonics of oceans and continents is not caused by the use of different methods on land and at sea, for the same worldwide distribution of seismic stations is used for all seismic studies.

The relation between sea floor spreading and continental geology has always been of interest, but no progress can be made until the nature of continental plate boundaries has been understood. For several reasons the Mediterranean region seemed to be a suitable area for this purpose. The entire region is seismically active, and many of the surrounding countries maintain good seismic stations. Also the outlines of the land and the marine geology of the area are known. Perhaps the most important reason was the general belief among land geologists that a large ocean, Tethys, once existed between Eurasia and Africa during the Cretaceous and early Tertiary time. The present Mediterranean is believed to be the remnant of this ocean. There was therefore some hope that the present tectonics of the area would demonstrate what happens when a trench attempts to consume a continent.

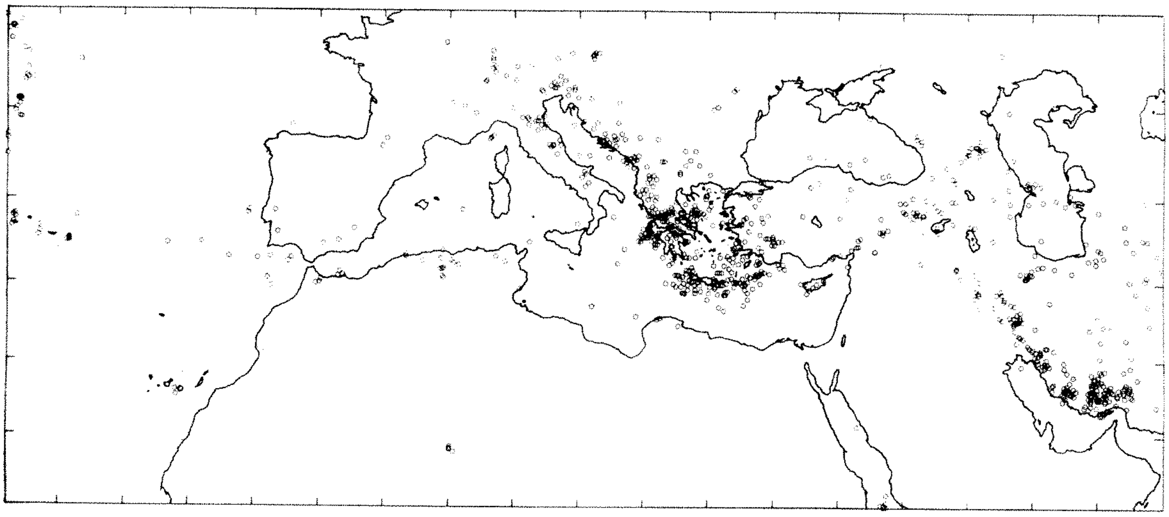

Fig. 1. Epicentres of all earthquakes with foci shallower than 70 km located by the Coast and Geodetic Survey between 1962 and 1967. The scattered activity in Eastern Turkey and Persia shows that the northward movement of Arabia is not taken up on a single fault. Six years' observations are not sufficient to show the North Anatolian Fault, though the Aegean plate is well defined. The plate boundary across North Africa is much less active than those further east, and continues into the Atlantic as the Azores–Gibraltar Ridge.

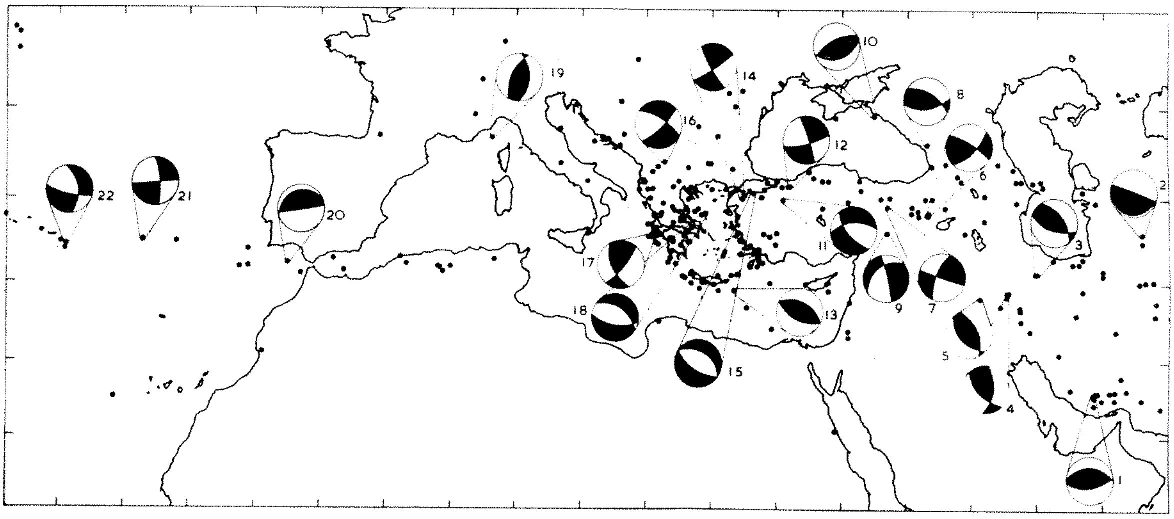

Fig. 2. Epicentres of earthquakes since 1925 whose magnitude was greater than about 6 are shown as solid dots. The focal mechanisms of some of these shocks are represented by balloons which show the lower hemisphere in stereographic projection with the compressional quadrants black. The North Anatolian Fault and the Azores–Gibraltar ridge and its eastward extension are more obvious than in Fig. 1, but otherwise the seismic activity resembles that of the shorter period.

This process is believed to produce wide belts of fold mountains.

Fig. 1 shows the region selected, which extends far beyond the boundaries of the Mediterranean Sea. The epicentres of earthquakes are plotted, taken from the United States Coast and Geodetic Survey between 1961 and 1967. It is clear from the seismicity (Figs. 1 and 2) that many small plates occur in the Mediterranean, but in two places, the Caucasus and the Azores–Gibraltar ridge, intervening plates are absent. These two special cases greatly simplify the problem of understanding the tectonics elsewhere. The seismicity of continental areas shows that such simple boundaries often exist[13], and it is essential to choose a region sufficiently large to include them. If, for instance, this study had been restricted to the Mediterranean itself it is doubtful if any progress would have been made.

There are now several methods of determining the position of plate boundaries and the magnitude and direction of the relative slip vectors between adjacent plates. Only epicentres and focal mechanisms of earthquakes are used in this study; thus the shape and direction of motion of the plates can be obtained, but not the rate of slip[5,8]. Figs. 1 and 2 show the position of the plate boundaries in this region. The seismicity associated with the Azores–Gibraltar ridge marks the junction between the African and the European plates, but east of Sicily the boundary is no longer sharp and the focal mechanisms do not reflect the motion between Europe and Africa. The focal mechanisms in Fig. 2 are in stereographic projection. They are projections of the lower hemisphere onto a horizontal plane, with the compressional quadrants shown as black regions. Thus right handed strike slip faulting gives a solution like 21 if the faulting is on an east–west fault, normal faulting gives a solution like 15, and overthrusts solutions like 13. Solutions like 11 and 17 are combinations of normal and strike slip faulting, and of thrust and strike slip faulting respectively. All solutions except 2, 4, 11, 17 and 21 were obtained by reading the vertical long period seismograms obtained from the World Wide Network of Standardized Stations. No such solutions could be obtained for the five earthquakes just mentioned because they took place before the network was installed. They were obtained by plotting all available readings in an equal area projection, then fitting nodal planes. About 100 solutions have now been obtained in this area, and will be published individually elsewhere. The plate boundaries and motions described account for most of these mechanisms, except those obtained for earthquakes on the Greek mainland. Here the deformation is rather complicated, and may be related to the motion of plates too small to be resolved by the seismicity. The solutions in Fig. 2 are some of the best obtained, and are chosen for this reason alone.

Only three plate boundaries of any importance enter the area, and therefore only three major plates are involved in the tectonics. These plates are the African, Eurasian and Arabian plates, and the boundaries between them are the Azores–Gibraltar ridge and its extension across North Africa, the Red Sea and the Alpide zone in Iran (Fig. 4). The line of earthquakes extending from Sicily to the Azores across North Africa out into the Atlantic following the Azores–Gibraltar ridge marks the boundary between the African and Eurasian plates. Several large earthquakes have occurred on this boundary, of which the most famous was the Lisbon earthquake of 1755. Two more recent earthquakes, in 1942 and 1969, were of magnitude 8 or more. The pole of relative motion between these plates has been calculated to be at 9° N, 46° W by Le Pichon (misprinted in his original paper). This pole requires the motion between Africa and Eurasia to be strike slip at the Azores, changing to overthrust south of Spain and in North Africa. Such motion agrees excellently with the fault plane solutions in Fig. 2, and with some other less good solutions in North Africa. Both the pole position and the fault plane solutions show that the motion between these plates is strike slip with a small amount of extension near the Azores. Because the pole of rotation is close to the area, the direction of motion changes rapidly going eastward along the Azores–Gibraltar ridge. The fault plane solutions show this change in more detail (Fig. 2), with the strike slip faulting changing to overthrusting in the Straits of Gibraltar and across North Africa. This remarkable variation is rare, and studies of this boundary are therefore of considerable interest. Also few examples of plate boundaries are known with slow consumption rates, and the features associated with such structures have not been studied in any detail. It is surprising that few intermediate depth earthquakes are associated with this boundary. Those beneath the Tyrrhenian Sea are unlikely to be directly related to the motion of Africa towards Europe because they are confined to a local area, and also because they are within a slab which dips west[14], or at right angles to the direction of motion of Africa.

The seismicity east of Sicily does not show whether the plate boundary between Africa and Europe extends across the south end of the Adriatic to join the Cretan Arc, or whether the motion is taken up by the activity in Italy and Yugoslavia. A dramatic change occurs in the eastern Mediterranean. The seismic activity of Albania, southern Yugoslavia, Greece and Turkey is very much greater than that of the western Mediterranean. Large earthquakes of magnitude greater than 7 are common, and are often associated with slip on major faults. Also the fault plane solutions become complicated (Fig. 2). This change is not the result of an active plate boundary joining that between Africa and Europe, nor is it the effect of the opening of the Red Sea[15]. The cause of this local increase in activity is the existence of two small rapidly moving plates, one containing the Aegean, part of Greece, Crete and part of western Turkey, the other containing most of Turkey and Cyprus. These plates will be called the Aegean and Turkish plates respectively. The motion between the Aegean and the Eurasian plate is shown by fault plane solutions such as 11, 14, 15 and 18. These solutions vary between strike slip and normal faulting. They therefore permit the fault plane to be distinguished from the auxiliary plane, and hence determine the slip vectors. These solutions show that the Aegean plate is moving towards the south-west relative to the European plate, producing extension and strike slip on the boundary between them. This boundary crosses from the Sea of Marmara across the northern Aegean, and probably reaches Greece between the island of Evvoia and the mainland. A deep trough exists in this part of the Aegean which reaches to a depth of 1,500 m and is associated with large magnetic anomalies[16]. The fault plane solutions suggest that this trough has been produced by extension of the crust, associated with large basic intrusions. Therefore from the point of view of plate tectonics this feature is a ridge. It is, however, very different from most oceanic ridges because the relative motion between the two sides is partly right-handed strike slip and partly extension, and also because the total extension is comparable to that of the East African Rift, rather than the major oceanic ridges. The present seismicity in the northern Aegean does not follow this trough, though the faulting is of the type which could produce it.

The southern boundary of the Aegean plate is moving south-west relative to the African plate, and is overthrusting the Mediterranean Sea floor. The direction of motion is given by solutions 13 and 17 (Figs. 2 and 4) and also by the existence of intermediate earthquakes beneath Greece and the southern part of the Aegean (Fig. 3). This plate boundary has several other features which show

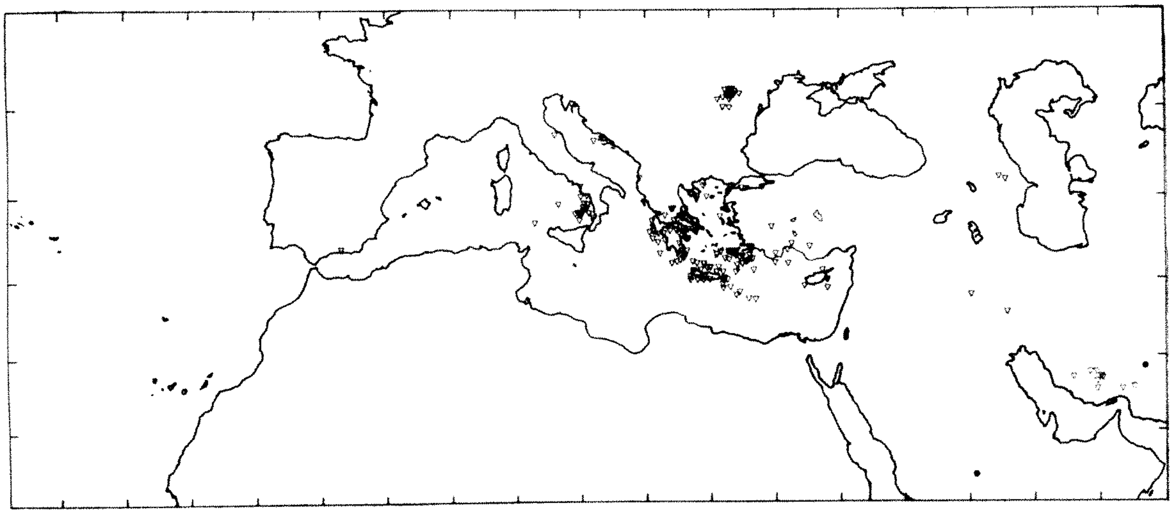

Fig. 3. Epicentres of all earthquakes with foci below 70 km depth which occurred since 1925. The active regions are localized and, if the concepts of plate tectonics are correct, mark the sites of consumption of lithosphere active during the last 10 m.y. (refs. 11 and 24).

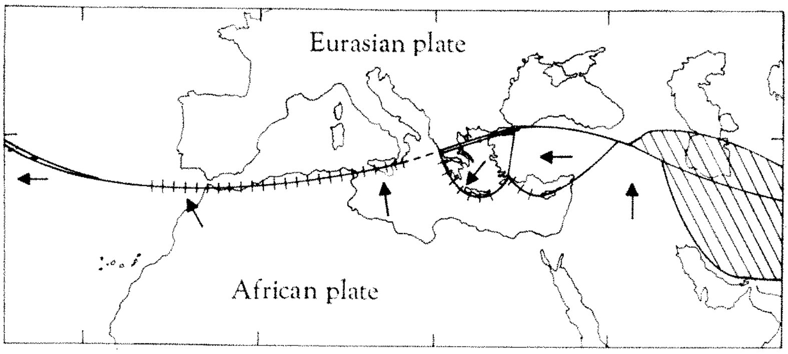

Fig. 4. Approximate positions of plate boundaries at present active, with arrows marking the directions of motion relative to the Eurasian plate. Boundaries creating lithosphere are shown with a double line, boundaries consuming plates with short lines at right-angles to them. The cross-hatched region in Eastern Turkey and Iran is seismically active throughout. Fault plane solutions here are all overthrusts (Fig. 2), and show that the crust is being thickened all over this region. Most major shocks within the cross-hatched area occur on major active faults mapped by Wellman[26] from aerial photographs and shown as solid lines.

that it is indeed a trench. Active and dormant andesite volcanoes occur in Greece and in several of the Aegean islands, of which Santorini is the most famous. Several surveys have detected a large negative free-air gravity anomaly[17,18] and deep depression in the sea floor extending from Zakinthos to Rhodes[19]. Also Ryan[20] has demonstrated the existence of thrust features in the seismic reflexion records of sediment deformation. All these observations show that the sea floor south of the Cretan arc is being overridden from the north-east and sinks beneath the Aegean Sea.

The seismicity of the Turkish plate and the fault plane solutions show that it is moving approximately due west relative to both Europe and Africa. The northern boundary of the plate is the North Anatolian fault, which is an active east–west right-handed strike slip fault whose slip rate has been estimated[21] to be 11 cm yr^{-1}. It is continuous with the northern boundary of the Aegean plate, which takes up the motion towards the west. Many earthquakes have occurred on this fault throughout northern Turkey, and many have produced surface displacements[22,23]. There is therefore no ambiguity between the fault plane and the auxiliary plane. The southern boundary of the Turkish plate is not well defined by the seismicity, and only one poor solution, No. 9, has been obtained on it. It seems to join the southern boundary of the Aegean plate south of south-west Turkey, and to run south of Cyprus into the Gulf of Alexandretta to join the North Anatolian fault east of Erzincan. The motion across this boundary is mostly left-handed strike slip, probably with some overthrusting in the south-west. The surface features and fault plane solutions associated with the North Anatolian fault show a striking change east of Erzincan. West of this point the fault is strike slip and has a surface trace which closely resembles that of the San Andreas. To the east, however, there is no distinct trace, and the fault plane solutions show a large component of thrusting, with the north overriding the south. Solutions in the Caucasus and further south show that the horizontal projection slip vector is north–south, and the fault plane solutions are overthrust. In this region the seismic activity is confined to a narrower belt than it is either to the east or west, though it is still spread over a wider belt than is common in the oceans. Thus in eastern Turkey, the Caucasus and western Iran the fault plane solutions show that Africa and Arabia are moving northwards, and though the motion is taken up on several faults, there are no small plates moving rapidly east or west. Thus the existence of the Anatolian fault and other large strike-slip faults is caused by the rapid motion of the Turkish plate westward. The geometry and motions of both the Turkish and Aegean plates are both complicated and surprising. A possible explanation depends on the difference in behaviour between continental and oceanic lithosphere. Continental material is too light to sink through the mantle, and therefore cannot vanish[24]. In parts of the Alpide belt such as Iran and the Caucasus, large thicknesses of continental rocks have accumulated, and considerable work must be done against gravitational forces to further thicken the continental crust. No such work need be done if oceanic lithosphere can be consumed, rather than continental lithosphere thickened. This is indeed the result of the motion of the Aegean and Turkish plates, which avoids further overthrusting in Turkey and instead consumes the Mediterranean Sea floor in front of the Cretan arc. Thus the present motions are such as to minimize the work which need be done to move the African Plate towards the Eurasian. This particular mechanism may also account for the existence of localized sources of intermediate earthquakes, such as those beneath Roumania and the Hindu Kush[13]. The earthquakes in these regions occur in slabs within the mantle which closely resemble those beneath island arcs[25]. These sources are puzzling because both are now overlain by continental crust, yet all other sources of intermediate earthquakes occur within a slab of what was originally oceanic lithosphere. The process at present taking place in the eastern Mediterranean is producing a localized source of intermediate earthquakes by rapid underthrusting beneath the Cretan arc. A similar mechanism could consume remnants of oceanic crust in other regions, such as the southern part of the Caspian Sea. This process is only possible if small plates of continental lithosphere are easily formed to consume oceanic crust in a limited region. It is therefore important to search for geological evidence for the previous existence of such plates in Roumania and the Hindu Kush.

The tectonics from the Caucasus to the eastern limit of the area appears to be considerably simpler than that further west. All fault plane solutions east of 44° E are overthrust, some with small components of strike slip faulting. Many solutions contained only one dilatational reading, always from a close station; and if the focus was taken to be in the mantle it was rarely possible to draw two orthogonal planes separating the compressions from the dilatations. Because several of the earthquakes in question produced surface breaks on faults[26,27], this difficulty is not caused by an explosive type of source mechanism but by refraction of the rays at the Moho or above. If the foci were taken to be in the crust, with a velocity of 6·8 km s^{-1}, all mechanisms could be described by a double couple source with the fault plane at right angles to the auxiliary plane. There is, however, at present little accurate information about the deep crustal velocity structure in this region, and therefore none of these mechanisms is well determined. In particular, most could have a larger component of strike slip than shown in Fig. 2.

All solutions in southern Russia and Iran show that this part of the Alpide belt is taking up the motion of India and Arabia towards Asia by overthrusting over a wide belt from southern Iran to the central Caspian. Most earthquakes are associated with the major active faults described by Wellman. The sense of displacement on surface faults may be used to determine the fault plane and the auxiliary plane, and hence to obtain slip vectors. The resulting vectors show that the motion direction varies little from 35° E over the entire region. This direction is little different from 15° E, the direction to be expected from the Arabia–Eurasia pole at about 25° N, 12° W. Thus the deformation of Iran and southern

Russia appears to be much simpler than that of the Eastern Mediterranean. The result of present activity in this part of the Alpide belt is to thicken the continental crust over a wide region. Though Gutenberg and Richter[28] show a few intermediate earthquakes in southern Iran, the deepest shocks with reliable depths from 1962 to 1967 were at about 100 km. There are not sufficient stations in this area to produce reliable depth estimates if, as seems probable, there are high velocity slabs still present in the mantle. Thus there is little evidence for any destruction of continental lithosphere throughout this region.

This account of the tectonics of the Mediterranean and Western Asia shows that the concepts of plate tectonics provide a useful description of continental tectonics. It also suggests that the boundaries of continental plates often may be complicated by the existence of small rapidly moving plates whose motion is not simply related to the motion of any of the major plates involved. A more complete account containing about 100 fault plane solutions on which this study is based is to be published elsewhere.

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Plate Tectonics of the Red Sea and East Africa

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The relative motion between the plates on each side of the East African Rift Valley can be obtained from the opening of the Red Sea and the Gulf of Aden. The calculated direction of relative motion agrees well with fault plane solutions for earthquakes north of the equator.

THE Red Sea and the East African Rift Valley were the first major features to be recognized as having been produced by extension of the Earth's crust[1,2]. Normal faulting along the sides of the Rift Valley during earthquakes shows that this motion still continues[3]. As the seismic activity of the Indian Ocean ridges is continuous with that of the East African Rift (ref. 4 and unpublished work of Fairhead), the African Rift served as a model for oceanic rifts. It was therefore studied in some detail because the significance of rifting in the oceans was not at first understood.

It is somewhat surprising that this situation is now reversed. Modern theories and oceanographic methods have been so successful that the evolution and tectonics of the ocean floor are now much better studied and understood than similar processes on land. It is therefore now possible to construct an outline of the movements involved in the formation of the Rift Valley from the history of the surrounding ocean basins. Such a reconstruction is attempted here, and the results are in general agreement with studies of the Rift Valley itself. Perhaps the principal result of this and other studies of this region is that, though the Rift is still of great interest, it is not of global importance. The discussion below demonstrates that the extension by which it was produced is everywhere less than 65 km, and probably took place in the last 20 million years. This motion is small compared with 3,000 km in the East Central Pacific during the same period, or even with 500 km in the North Atlantic.

The seismicity of the Red Sea, Gulf of Aden and the East African Rift (Fig. 1*a*) shows that three plates meet at the south end of the Red Sea. These plates we will call the Arabian, Nubian and Somalian Plates, and will only consider the relative motions between these plates in Africa north of the equator, in the Red Sea and Gulf of Aden, and in Egypt, Jordan and Israel. If the motions between the Arabian plate and the Somalian and Nubian plates respectively can be determined, the motion between the Somalian and Nubian plates may be calculated from the postulates of plate tectonics[5,6]. The results of such calculations are shown in Fig. 1*b*, where the heavy double lines mark the three extensional plate boundaries and the arrows show the relative motion between the plate on which the arrow lies and the adjoining plate. A junction between three ridges is possible without compression of any of the plates involved.

The motion between the Arabian and Somalian plate was first determined by Laughton[7] from the strike of transform faults in the Gulf of Aden. He used them to fit the coast of Africa to that of Arabia, and showed that the geological structures on each side then matched remarkably well. The seismicity and fault plane solutions for earthquakes[3,4] in the Gulf and the magnetic lineations agree with the direction of motion proposed by Laughton.

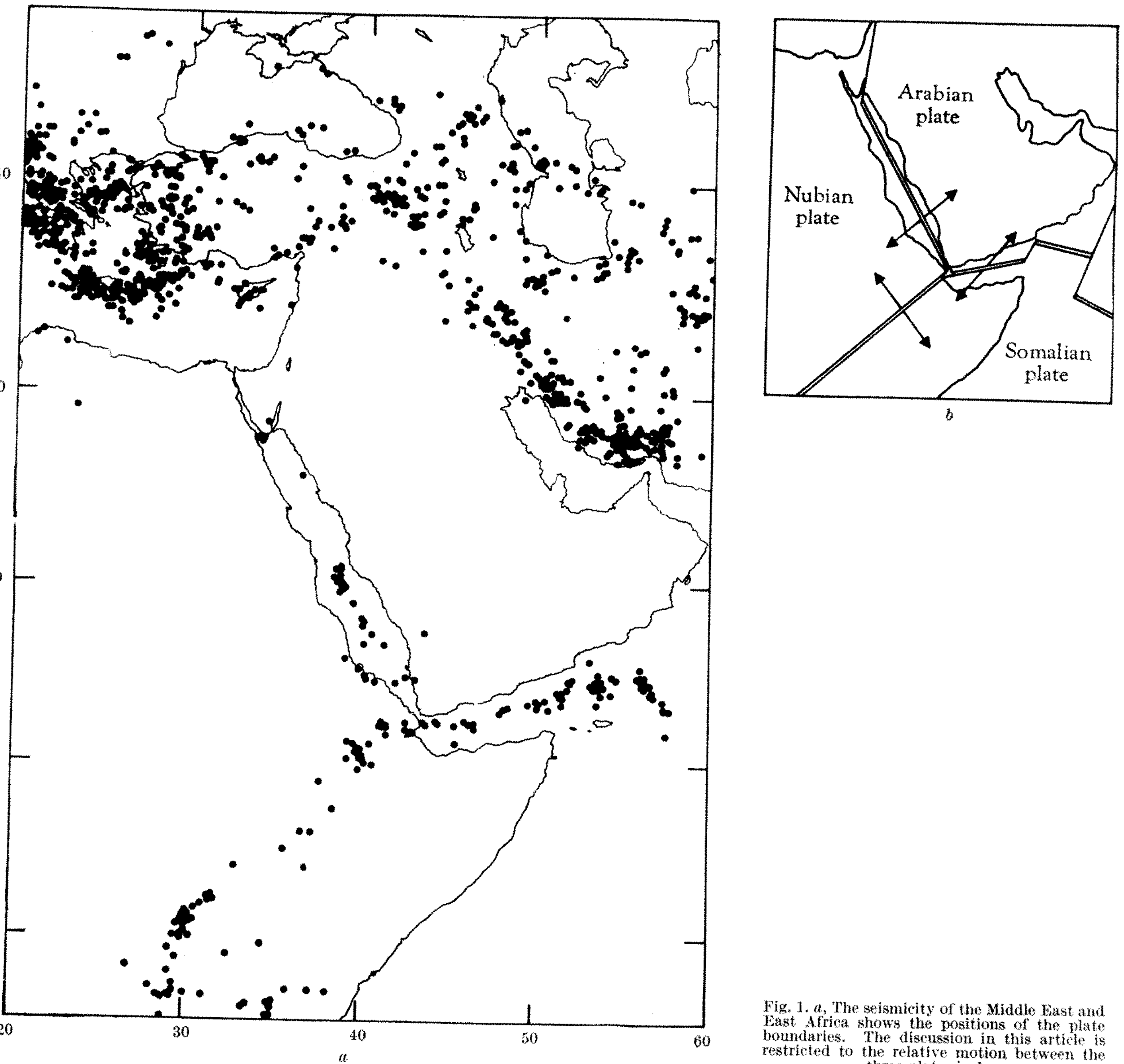


Fig. 1. *a*, The seismicity of the Middle East and East Africa shows the positions of the plate boundaries. The discussion in this article is restricted to the relative motion between the three plates in *b*.

The pole of rotation of the Arabian plate relative to the Somalian plate may be obtained either from the transform faults and magnetic lineations[9], or by fitting the 500 fathom contours on each side of the Gulf. The pole positions from the two methods are within a degree of each other, and the pole from the fitting at 26·5° N, 21·5° E, with a rotation angle of 7·6° is used throughout this discussion. The resulting fit is shown in Fig. 3, and demonstrates how accurately the two sides match. Neither Socotra nor Mount Error, however, both of which are probably continental fragments, fit back onto Arabia. The separation of Africa must therefore have involved a progressive penetration of the Carlsberg Ridge into the continent.

In contrast to the Gulf of Aden, the structure and evolution of the Red Sea are much more controversial. Though Wegener[9] noticed that the coast lines on each side fitted together, it seemed unlikely for several reasons that the whole of the Red Sea was underlain by oceanic crust. Bathymetric surveys showed that the sea floor consisted of shallow marginal seas on each side of a deep main trough. Also gravity surveys had shown almost no free air anomaly. These observations suggested to Girdler[10] that the main trough was underlain by continental rocks and that only the axial trough was underlain by sea floor. This suggestion was supported by measurements of the magnetic anomalies, which are of large amplitude above the axial trough but almost absent elsewhere.

These arguments became less convincing when the Red Sea was drilled in search of oil, and thicknesses of up to 5 km Miocene evaporite were found[11]. This result suggested that the marginal seas were directly underlain by thick beds of evaporite and not by continental crust. Seismic results also strongly supported such a model. Tramontini and D. D.[12] shot a large number of seismic refraction lines parallel to the axial trough and showed that the marginal seas consisted of 3 to 5 km of material with a seismic velocity of 4·3 km s^{-1} overlying the basement. Because the basement had a velocity of 6·6 km s^{-1} they suggested that it was oceanic crust rather than subsided continental material. They also showed that such a structure is compatible with the gravity observations. If a large part of the Red Sea is indeed underlain by oceanic crust, as these observations suggest, the only remaining difficulty is the absence of magnetic anomalies of substantial size in the main trough. Calculated anomalies for this latitude and spreading rate have an amplitude of 100 γ or less, however, and anomalies of this magnitude could be present in parts of the seas. Thus there is now considerable evidence that the entire Red Sea trough has been formed by the movement of Arabia away from Africa, and therefore that the pole and rotation angle

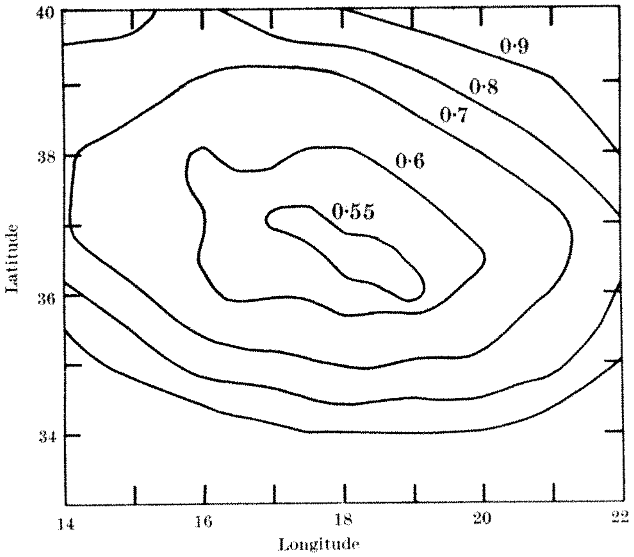


Fig. 2. Contours of the total misfit area (overlap and gap) in square degrees for the Red Sea as a function of the latitude and longitude of the rotational pole. The rotation angle is adjusted at each point to minimize the misfit.

for the separation can be obtained by fitting together the coast lines. It is fortunate that this is the case, because all bathymetric contours are governed by coral reefs and by salt accumulations, and therefore cannot be used for this purpose.

The two coast lines north of 15° N were fitted by choosing a latitude and longitude for the rotation pole, then rotating one until the total misfit area, without respect for its sign, was minimized. The procedure was then repeated for a different choice of pole position until a contour map of the misfit area as a function of pole position could be drawn. All programmes used rotation matrices and vectors, which are both easier to use and more economical of machine time than programmes which use spherical geometry. The total area of misfit, rather than the fitting criterion of Bullard, Everett and Smith[13], was used to avoid difficulties which occur with their scheme if parts of the coast lines are small circles about the relative rotational pole, and also to avoid the weighting problems which occur when the coasts are close to the rotation pole. In this case, however, the same pole at 36·5° N, 18° E, off western Greece, is obtained by both methods (Smith, personal communication). The contours of the misfit area as a function of pole position (Fig. 2) show that the minimum is a linear trough surrounded by a rather steeply sloping bowl. No local minima were found, and the shape of the surface suggests that any that may exist must occur within the narrow trough, and are therefore unimportant.

Fig. 3 shows the resulting fit of the Red Sea coast lines. As previous authors have observed, the fit is remarkably good, and it is hard to distinguish the rotated coast line of Africa from that of Arabia. Because it is difficult to imagine any geological process other than splitting which could produce such a remarkable correspondence between opposing coast lines, this fit therefore also suggests that the whole of the Red Sea trough has been produced by the separation of Arabia from Africa. At the south end of the Red Sea the two coast lines converge, and therefore overlap when the northern part is closed. This discrepancy suggests that Afar depression has been largely created from mantle materials during the separation, and recent aeromagnetic evidence[14] has supported this view.

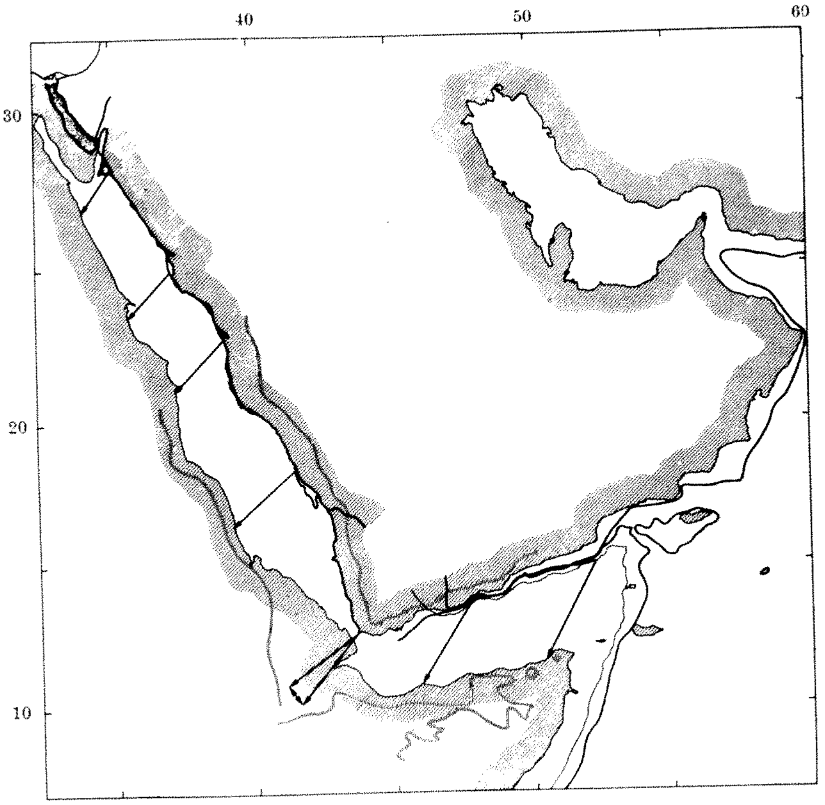


Fig. 3. The fit of the Red Sea coast lines and the 500 fathom contours in the Gulf of Aden. The arrows show the motion of the Nubian and Somalian plates relative to the Arabian plate during the opening of the Red Sea and Gulf of Aden. The difference between them gives the motion on the East African Rift. The narrow cross-hatched lines mark the 1,000 m escarpment on land.

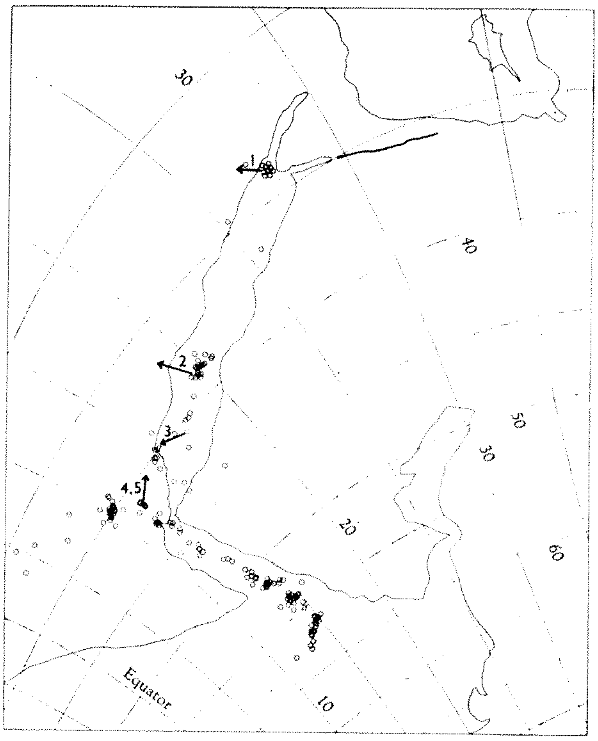
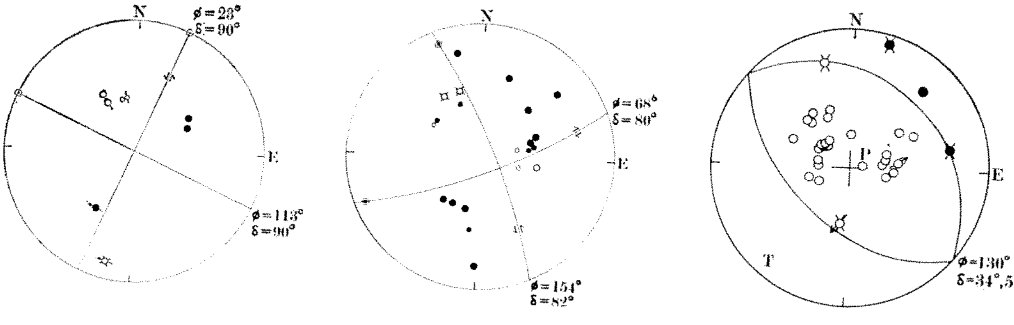


Fig. 4. Oblique Mercator projection of the Red Sea with axis 36·5° N, 18° E, the pole for the best fit of coast lines. The three slip vectors from the fault plane solutions in Fig. 5 are approximately horizontal and parallel to the top and bottom of the figure. The instantaneous motion is therefore along small circles with respect to the finite rotational pole.

The pole determined from the fit of the coast lines is not necessarily the present instantaneous pole of rotation between the Arabian and Nubian plates, nor need the motion between the two have been steady. There is, however, some evidence that neither the relative pole nor the rotation pole between the two plates differs greatly at present from the mean pole. Magnetic anomalies at 16° N on the axis of the Red Sea suggest that the spreading rate is about 1 cm yr^{-1} on each side[15]. If the separation occurred in the Miocene, or about 15 million years ago, and drift were continuous, this rate of drift would produce a separation of 300 km, or close to that observed. It must, however, be admitted that the identification of anomalies in this region is somewhat in doubt.

Fault plane solutions for earthquakes in the Red Sea permit an independent test of the accuracy of the instantaneous pole position, though not of the spreading rate. Fig. 4 is a map of the Red Sea and surrounding regions in an oblique Mercator projection with the relative rotational pole as the Mercator axis[5]. Because the relative motion between plates must always take place along small circles about the relative rotational pole, this Mercator projection has the property of making all rotations about this pole become translations along straight lines parallel to the top and bottom of the map. It is therefore possible to close the Red Sea by sliding one coast horizontally against the other with no rotation. The present motion between the Arabian and Nubian plates may be obtained from fault plane solutions of earthquakes in the Red Sea. Though these are rarely large enough to permit reliable solutions to be obtained, three such earthquakes have occurred in this region since the World Wide Long Period Network was installed. One of these, the slip vector of which is numbered 3 in Fig. 4, was obtained by Sykes[3]. This solution was not well controlled (Fig. 5) and the direction of the slip vector is uncertain by perhaps 30°. Fig. 5 shows solutions 1 and 2, both obtained from reading the long and short period records, from standard stations alone. Solution 1 shows normal faulting, and it is impossible to decide which is the fault plane and which the auxiliary. Fortunately, no such distinction is necessary if the horizontal projection of the slip vector alone is required, because it must lie at right-angles to both planes. The location of this earthquake at the south end of the Gulf of Suez means that it is impossible to decide whether it is caused by the separation of Arabia from Africa or by the movement of Sinai from Africa. Because, however, the difference between the two slip vectors is within the experimental error for strike of the slip vector, solution 1 may be used to determine the motion between the Arabian and Nubian plates. Solution 2 is a strike slip solution, and it is therefore necessary to determine which plane is the fault plane. If the plane marked "fault" in Fig. 5*b* is chosen, the resulting slip vector (Fig. 4) is consistent both with the direction of opening of the Red Sea and with the slip vector from solution 1. Neither remark is true for the other possible choice, which is therefore excluded. Though the slip vector for solution 2 is consistent with the direction of spreading, the mechanism is peculiar. There is no feature on the sea floor close to the earthquake to suggest that the ridge is offset by a transform fault near this earthquake. What is perhaps even more surprising is that the mechanism corresponds to right-handed slip on a fault, or a left-handed offset of the ridge axis. This is in the opposite sense to that suggested by the general trend of the Red Sea relative to the pole of rotation of Arabia relative to Africa (Fig. 4).

These three solutions are consistent with the hypothesis that the present pole for the opening of the Red Sea is the same as the pole obtained from fitting the coast lines, though none of these solutions is sufficiently good to provide a rigorous test of this hypothesis. Until more



a *b* *c*

Fig. 5. *a*, Fault plane solution for earthquake 3 in Fig. 4 on November 11, 1962. Reproduced through the courtesy of L. R. Sykes. *b*, Solution for earthquake 2 on March 13, 1967, at 19·7° N, 38·9° E. *c*, Solution for earthquake 1 on March 31, 1969, at 27·5° N, 33·8° E.

earthquakes occur, the fitting pole provides the best instantaneous pole for this area.

No use has yet been made in this discussion of the work done by Quennell[16], Freund[17,18] and others on the evolution of the Dead Sea rift. Their careful work has established that there has been close to 110 km left lateral motion on the Dead Sea fault system since the Miocene. The eastern margin of the Dead Sea Valley is shown as a heavy line in Fig. 4. As it is not parallel to the top and bottom of the figure it cannot be a transform fault between the Arabian and Nubian plates. This result is supported by the magnitude of the displacement on the Dead Sea fault, which is 80 km less than the 190 km required at this latitude to open the Red Sea if the present shore lines were once in contact. This difference must be taken up on some other structure, and the only possible choice is the Gulf of Suez and the low lying land to the north. The whole region is a large graben which has been filled with up to 3 km of evaporites[19]. The basement which occasionally outcrops is Pre-Cambrian in places, and not oceanic crust. It is possible to reconstruct the north end of the Red Sea by rotating Africa towards Arabia to close the Red Sea, then sliding Sinai 110 km northwards along the Dead Sea rift. The resulting reconstruction closes the Gulf of Suez (Fig. 3). Fig. 6 shows the motions of Arabia and Africa relative to Sinai in more detail, and demonstrates that the extension required across the Gulf of Suez is between 60 and 90 km. The reconstruction leaves a gap of 20 to 30 km between exposures of unfaulted Pre-Cambrian basement on each side of the Gulf. If the extension occurred by normal faulting over a region perhaps originally 50 km wide, crustal thickness would decrease from 30 km to 15 km. A difference in elevation of about 3 km would still remain after isostatic compensation. This difference is comparable with the thickness of salt found in bore holes. Girdler *et al.*[20] have suggested a similar mechanism to account for the gravity field in East Africa. They, however, suggested that the process occurred by necking rather than by faulting. Rocks fail by brittle fracture at the temperatures which occur in a Pre-Cambrian crust, so extension should cause normal faulting. There is no obvious reason why extension in the Gulf of Suez takes place by this process rather than by the formation of ocean floor. Perhaps the activation of many normal faults depends on the existence and orientation of existing basement faults. The long history of activity in the Gulf of Suez suggests that many such faults must have existed.

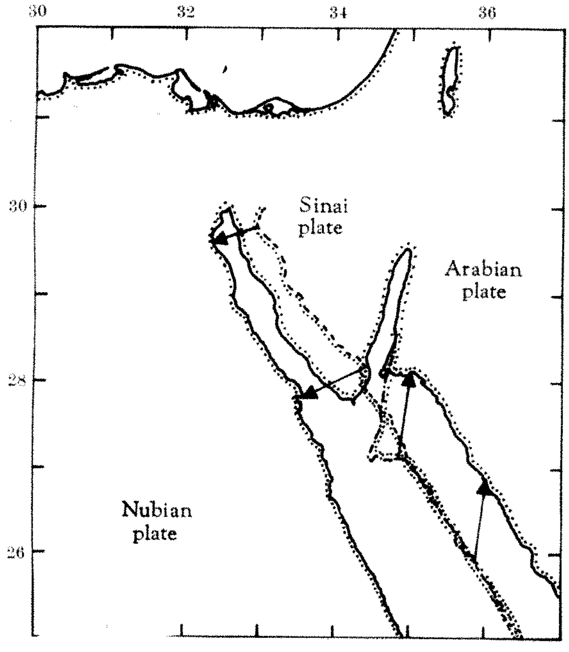


Fig. 6. Relative motion between the Sinai plate, taken to be fixed, and the Arabian and Nubian plates is shown by the arrows.

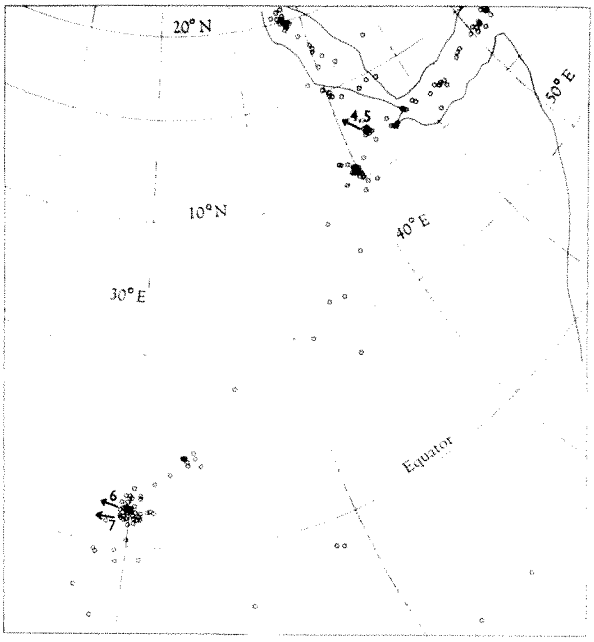


Fig. 7. Oblique Mercator projection of East Africa with axis at 8·5° S, 31° E. As in Fig. 4, the slip vectors are approximately parallel to the small circles about this pole which form the top and bottom of the figure.

This discussion of the opening of the Red Sea shows that there is no conflict between geological evidence and the evolution suggested. The poles of opening and rotation angles for the Red Sea and Gulf of Aden may therefore be combined to obtain the pole and angle of rotation between the Nubian and Somalian plates. Because the relative rotation angles are all small, a good approximation to the pole for the rift may be obtained by adding the other two rotations as vectors. The resulting pole is at 8·5° S, 31·0° E, with a rotation angle of 1·9°, and the opening on the rift that has taken place varies from 65 km in northern Ethiopia to 30 km in Kenya. It is doubtful whether the Rift Valley south of the equator has opened about this pole because the seismic belt which runs between Madagascar and the mainland probably joins the Rift Valley in Tanzania. These extensions are of the same order of magnitude as those estimated from field geology[21].

The mean pole and rotation angle need not coincide with the instantaneous pole for the rift. As in the case of the Red Sea, however, the coincidence of the two poles may be tested by using fault plane solutions. Fig. 7 is a map of the seismicity of the Rift Valley in Mercator projection with the Nubian–Somalian rotational pole as axis. The slip vectors from four earthquakes on the Rift and its extension, numbers 4, 5, 6 and 7, are approximately parallel to small circles, and therefore suggest that the present pole is close to the mean pole of opening.

Solutions 4 and 5 (Fig. 8) both show strike slip faulting. There is, however, no ambiguity about which nodal plane is the fault plane, because the surface displacement which accompanied these earthquakes was along a fault striking N 40° W (ref. 22). Solution 6 shows normal faulting and was published by Sykes[3]. Solution 7 was published by Banghar and Sykes[23], and was an aftershock of 6. If its mechanism is taken to be the same as 6 the slip vector is well determined. Earthquake 6 also produced a surface break which again decides the orientation of the slip vector. No other solutions using long period seismograms have been discovered. Solutions 4, 5, 6 and 7 show that

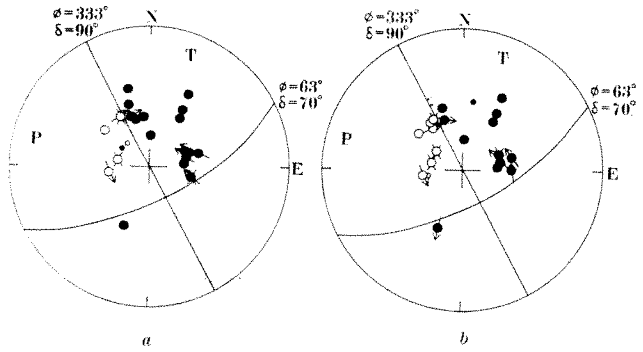


Fig. 8. *a*, Fault plane solution for earthquake 4 in Fig. 7 on March 29, 1969, at 12·0° N, 41·2° E. *b*, Fault plane solution for earthquake 5 in Fig. 7 on April 5, 1969, at 12·2° N, 41·3° E. The fault plane solution for earthquake 6 was published by Sykes[3], that for 7 by Banghar and Sykes[23].

the strike of the horizontal projection of the slip vectors is consistent with the pole obtained here.

The Mercator projection in Fig. 7 suggests various interesting possibilities concerning the structure and evolution of the Rift Valley. From the equator to about 5° N the seismically active region is offset in a right-handed sense, and therefore earthquake mechanisms in this region should show a combination of left-handed strike slip and normal faulting. Between 5° N and the Afar depression the motion vector is almost at right-angles to the seismic zone, and therefore normal faulting should be dominant. It is, however, surprising that the two solutions 4 and 5 for earthquakes within the Afar depression (Fig. 8) are consistent with the pole obtained for the Rift further south, and therefore that the boundary between the Arabian and Nubian plates does not cross the Afar depression but joins the Rift boundary east of the Gulf of Tadjura at the west end of the Gulf of Aden. The earthquakes, the solutions of which are shown in Fig. 8, must therefore lie on a right-handed transform fault joining the Rift Valley to the Gulf of Tadjura.

If the position of the ridge axis jumped from the western side of the Danakil horst to its present position in the Straits of Babel Mandeb a piece of what was the Arabian plate would be left attached to the Nubian plate. These speculations could therefore account for both the seismicity and the existence of the Danakil horst[7]. This elevated region in the Afar depression consists of Jurassic and older rocks similar to those of Arabia, to which it was presumably once attached. Further studies of the seismicity of the Afar depression should provide a test of this hypothesis, which is only consistent with the seismicity in Fig. 1 if a transform fault crosses the northern part of the Afar triangle.

This study shows that the concepts of plate tectonics can be useful where plates are separating at millimetres a year, and the total relative motion of which has been between 30 and 300 km. These rates are at least an order of magnitude smaller than those of most major plate boundaries in oceanic regions. This success is somewhat surprising, and may be due to the exceptional rigidity of the Pre-Cambrian shield areas cut by these active structures. This study also strongly suggests that the tectonics of some parts of continents can be described by the theory that has been so successful beneath the oceans. Several interesting features of this area deserve further study. Mount Error and Socotra do not fit against Arabia when the Gulf of Aden is closed, and therefore were broken off from Africa before the Arabian plate. The surrounding sea floor should have recorded the processes which take place when an existing ridge extends beyond its limiting transform fault, which in this case was probably the Owen fracture zone.

If the suggested evolution of the Afar depression is correct, this area contains one of the few ridges which is known to have jumped and the only such example yet discovered on land.

Another remarkable feature of the region is the trend of the magnetic anomalies in the Gulf of Aden[24]. The magnetic lineations are not at right-angles to the transform faults, and therefore the ridge which formed them was spreading obliquely to the motion direction between plates. Few other examples of such ridges are known. Several rather unconvincing suggestions have been put forward to explain why most ridges and magnetic lineations are at right-angles to their transform faults. Studies of the Gulf of Aden might resolve this problem.

Finally, we wish to point out two misunderstandings which have appeared in several previous papers on this region. The first involves rotations of plates on a sphere. Various authors have maintained that the opening of the Red Sea can be described by a translation northwards combined with a rotation, often taken to be a pole in Sinai. This description of the motion is incorrect, because no translations are possible on a spherical surface. These authors are therefore producing the displacement by two successive rotations. We prefer to use one rotation. Furthermore, any attempt to obtain a pole for the opening of the Rift by the method used here requires the single pole and rotation angle which open the Red Sea, and cannot be obtained from either of the other two poles alone.

The other confusion which has occurred involves the relationship between the opening of the Red Sea and Gulf of Aden and the active Alpide belt to the north. Fig. 1 shows the seismicity recorded by the United States Coast and Geodetic Survey between 1961 and 1968. It is clear that earthquakes originating in Iran, Turkey and Greece during this interval are much more frequent than those in the Red Sea and Gulf of Aden. Estimates of the slip rate between the plates in Turkey and Iran vary from 10 to 20 cm yr^{-1}, or more than an order of magnitude greater than those involved in the Red Sea. Also the high seismicity of Iran continues westward into Turkey and is unaffected by meeting the northward extension of the Dead Sea rift. Thus crustal generation between Arabia and Africa is quite unable to account for the seismicity of the region to the north.

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Rocket UV Flash Spectra from the Solar Eclipse of March 7, 1970

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Flash spectra of the total solar eclipse throughout all its phases have been obtained in the extreme ultraviolet for the first time.

AN Aerobee 150 rocket was launched at 18 h 36 m 15 s UT on March 7, 1970, into the total solar eclipse then passing over the established NASA launch range at Wallops Island, Virginia. The vehicle successfully completed its programmed pointing manoeuvres and, at 18 h 39 m 32 s UT, entered the umbra at an apogee of 142 km. A sequence of fifty spectra were recorded over a period of 180 seconds in the range 850 Å–2150 Å. The spectra are spatially resolved and show images of the eclipse in each spectral line. Each frame refers to the particular phase of the event as seen from the rocket at the time of exposure. The observed time sequence of the eclipse is determined by the dynamics of the Sun–Moon–rocket system and the fifty frames cover a range starting from before second contact, that is, the point of total obscuration of the visible disk, to near mid-totality. This article is a summary of the experiment.

The ideal trajectory for this type of experiment is one that is parallel to the direction of motion of the eclipse shadow and central within it. Within the constraints imposed by the vehicle performance and the location of this eclipse path relative to the range, the optimum configuration for the present experiment was determined and involved a trajectory approximately at right angles to the line of motion of the 120 km wide shadow cone. As the basic objective of the flight was to record the ultraviolet flash spectrum of the chromosphere, the conditions of launch were chosen, within the constraints, to yield second contact at rocket apogee and so minimize the effects of atmospheric absorption. Further factors that were considered in determining the final conditions of flight were: (i) a minimum apparent scan rate of the lunar limb, (ii) a minimum in apparent rotation of the line of centres throughout the data acquisition sequence and (iii) a minimum dependence on the dispersion probabilities of the Aerobee vehicle. A preliminary flight reconstruction shows that the chosen nominal was closely achieved, second contact occurring only seconds before rocket apogee.

The operational part of the rocket eclipse programme at Wallops Island was run by NASA, which also furnished the STRAP III pointing control used to orient the payload to the eclipsed Sun. This inertially referenced triaxial pointing control, using rate integrating gyros and assisted by an optical eclipse sensor, successfully manoeuvred and acquired the eclipsing Sun at an early stage of the flight. Throughout data acquisition the maximum pointing excursions in pitch and yaw were ± 10 arc s, with mean body rates of 5 arcs per second. There was a pointing null for the several critical frames taken around second contact with body rates less than 2 arcs per second. As a result, these data are limited only by optical aberrations and film grain.

Alternate exposures of 0·2 and 1 s were taken throughout the sequence of fifty frames, using an absolutely calibrated $f/10$ one metre normal incidence Wadsworth spectrograph. After this sequence of exposures and before re-entry, the camera and payload section were sealed and pressurized to two atmospheres. The payload then made a parachute return within visual distance of the recovery vessel, the aircraft carrier USS Guam. Prompt recovery permitted film development to begin at 23 h 30 m UT. The data are recorded on Kodak 101–01 film and are of excellent quality. The dispersion is 16·6 Å mm^{-1} and the solar images are 4·5 mm in diameter.

A preliminary flight reconstruction shows that the maximum apparent angular rate of the lunar limb at second contact is 0·36 arc s per second. This yields an apparent angular velocity for the Moon of 1·30 arc s per frame.

Second contact occurs close to the twentieth frame in the time sequence, at which point the chromospheric flash spectrum is clearly resolved. The sequence then progresses steadily to a point close to equal mid-totality resulting in spectra entirely dominated by the corona. Several prominences and a plage region are clearly visible in Lyman α and their spectra are isolated and spatially resolved throughout the whole sequence.

Figs. 1*a* and *b* show a part of the total wavelength range between 1100 Å and 1400 Å selected from two of the fifty frames. Fig. 1*a* corresponds to a few seconds after second contact while Fig. 1*b* is much later in the sequence and is close to mid-totality. These two frames illustrate many of the features shown in the complete data. The Lyman α images are of particular interest for one can observe structure and emission out to 1·3 solar radii. This is well beyond the emission from the coronal forbidden lines of FeXII at 1242 Å and 1350 Å, also clearly visible in the figure.

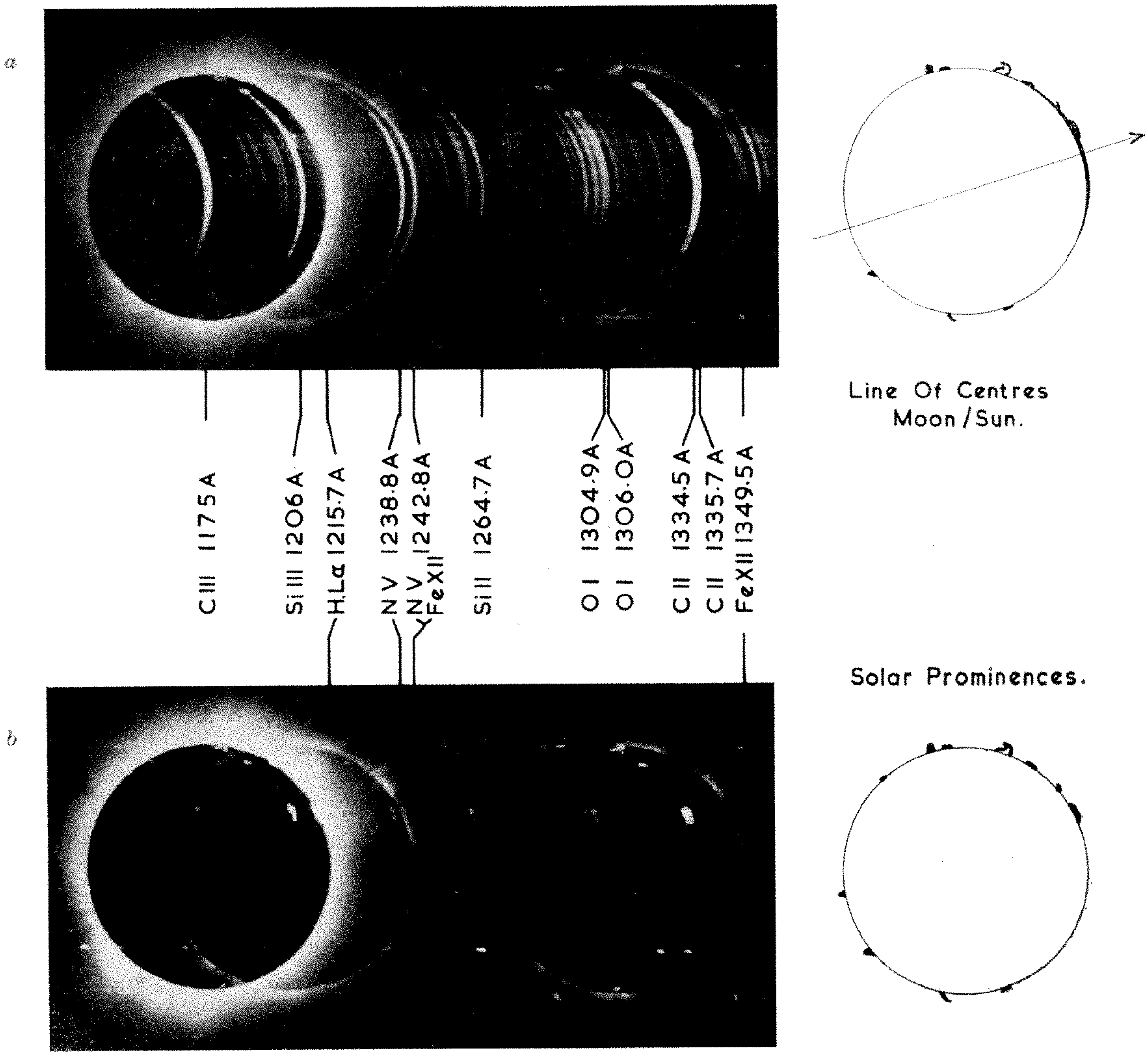

Fig. 1. Flash spectra of the total solar eclipse of March 7, 1970, covering a wavelength region near Lyman α for phases *a* just after second contact and *b* near mid-totality. The wavelength scale and identifications refer to the right-hand side of the lunar limb. Several limb prominences are clearly seen in Lyman α and a key is given in the sketches to the right which have been traced from the originals. The apparent lunar diameter is 72 arc s greater than that of the Sun. Exposure *a* shows a spectrum dominated by the chromosphere near the contact point whereas exposure *b* is mainly a spectrum dominated by the corona. Note the prominence spectra clearly isolated and the extent of Lyman α into the corona. Regions of coronal activity are seen most clearly in the forbidden lines of FeXII. The complete data consist of fifty frames covering a wavelength range 850 Å–2150 Å extending from a phase before second contact to mid-totality.

Preliminary estimates allow radiative recombination, electron excitation and dust scattering (F Corona) to be discarded as possible mechanisms to explain the observed intensity of coronal Lyman α. Thomson scattering can also be eliminated by noting the sharp edge of the lunar disk image, because this mechanism, if present, would result in a Doppler broadening of approximately 30 Å half width. We conclude that the observed intensity is due to resonance scattering of the intense eclipsed Lyman α radiation by residual neutral hydrogen atoms in the corona. Although the relative concentration of neutral hydrogen at a coronal temperature of 2×10^6 K is only about 10^{-7}, the high abundance of hydrogen and the high flux of the Lyman line from lower levels are sufficient to produce the observed intensity within the errors of this early calculation. This "Lα corona" therefore reflects the distribution of neutral hydrogen and has an intensity in the inner corona of the order of 10^{-3} of the low lying chromospheric Lyman α.

We gratefully acknowledge the help of many colleagues in the participating groups. The success of this experiment was made possible by the outstanding contribution of the NASA personnel in the Rocket Sounding Branch of the Goddard Space Flight Center and at the Wallops Island Missile Range. The extensive reduction and analysis of these data are currently under consideration by the participating groups.

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Prediction of the Coronal Structure for the Solar Eclipse of March 7, 1970

ON March 3, I made a prediction of the coronal structure of the March 7, 1970, eclipse of the Sun. The model employed is similar to that used for the prediction of the coronal structure at the eclipse of September 22, 1968, and details of the method are described in ref. 1. Observations of this eclipse seem to support[2] the model: "there is a general similarity between the two drawings" (prediction and observation)[3], and a sketch of the observations "agrees well with the prediction; had Schatten drawn his streamers more nearly radial, the agreement would have been almost perfect"[4].

A "source surface" at one solar radius above the photosphere was used in the present prediction, rather than one at 0·6 solar radii as used in the earlier work, to allow for the more active Sun. Comparisons of calculations obtained with this newer value at the end of 1968 agree more closely with observations of Faraday rotation from Pioneer 6 that provided a measurement of the coronal magnetic field (unpublished work of Stelzried *et al.*). Altschuler and Newkirk[5] use a zero-potential surface at 1·5 solar radii above the photosphere for comparisons with the solar eclipse of November 1966. I agree that the method of Altschuler and Newkirk for calculating coronal fields is superior, in principle. I have not had time to incorporate the improvements suggested by these authors into the present work. In practice, however, it is my opinion that uncertainties in the measured photospheric vector magnetic field and in accounting properly for the effects of the coronal plasma outweigh the differences in the two computational techniques. The effects of sunspot magnetic fields, as well as the photospheric magnetic field, were included in this analysis in accordance with Altschuler and Newkirk's work.

Fig. 1 is a prediction made on March 3 of the structure of the March 7, 1970, eclipse of the Sun (total over Mexico, the United States of America and Canada). Solar magnetic observations for this eclipse prediction were terminated on February 26, 1970, due to poor weather conditions in Pasadena, California. A list of the predicted coronal features within 60° of latitude from the solar equator is as follows. They are listed with their position angles (counterclockwise from the north pole) in approximate order of decreasing brightness and certainty: bright features (condensations) at 288° and 95°; streamers from 100° to 120° overlying coronal arches located at 100° and 110°; helmet streamers from 30° to 70° centred on 50° and from 285° to 310° centred on 302°; coronal arches located at 75°, 292° and 278°; broad streamers without arches from 235° to 270° and from 120° to 150°; narrow streamers possibly with greater radial polarization at 235° and at 145°.

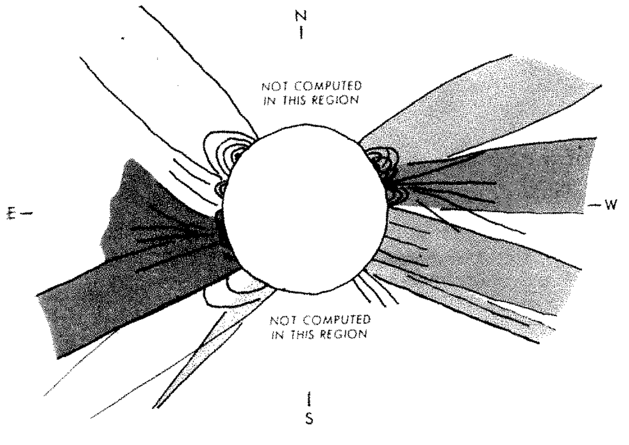

Fig. 1. Eclipse prediction drawn March 3, 1970.

The March 7, 1970, solar eclipse will probably be somewhat brighter and more elliptical in appearance than the September 22, 1968, eclipse (total over Siberia). This results from a greater number of regions of solar activity appearing in the lower latitudes following sunspot maximum.

I thank Dr John M. Wilcox and Dr Robert Howard for their help. This work was supported in part by the US Office of Naval Research, by the US National Aeronautics and Space Administration and by the US National Science Foundation.

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Indigenous Lunar Methane and Ethane

RECENT analyses[1,2] of Apollo 11 lunar samples have shown that CH_4 (up to 6 $\mu g\ g^{-1}$) and other gaseous hydrocarbons, ranging from C_2 to C_4, are released on treatment of the fines with aqueous HCl, H_3PO_4 or HF. It was uncertain whether these hydrocarbons were formed by acid hydrolysis or were present as such. We have now shown that methane and ethane arise from both sources.

Samples of Apollo 11 lunar fines (sample 10086·19 bulk fines D) have been examined in two ways; first, by crushing the sample *in vacuo* and examining by mass spectrometry the gases released and, second, by treating the sample with DCl and analysing the isotopic compositions of the methane and ethane. The analytical procedures have been described in detail elsewhere[2,3].

An aliquot of the sample was pulverized by shaking for 16 h in an all-glass ball mill after a vacuum degassing procedure (150° C, 10^{-6} torr, 30 min) to remove adsorbed terrestrial gases. The gases released by crushing included the rare gases (He, Ne and Ar), with Ne and Ar in the lunar isotopic abundances previously measured at the Lunar Receiving Laboratory[4]; CH_4 (1 $\mu g\ g^{-1}$) and C_2H_6 (0·1 $\mu g\ g^{-1}$) were also released (Table 1). A blank experiment conducted with the mill alone revealed only trace quantities of atmospheric gases. In a second experiment, D_2O (99·8 per cent, *c*. 1 ml.) was added to an aliquot of the sample in the ball mill and evaporated under vacuum (150° C, 10^{-6} torr). This procedure was repeated four times in order to exchange, as far as possible, all protonated species (particularly H_2O) with deuterated analogues.

Table 1. HYDROCARBONS AND DEUTERATED HYDROCARBONS RELEASED BY VACUUM CRUSHING AND DCl TREATMENT OF APOLLO 11 FINES

		Quantities of gases released ($\mu g\ g^{-1}$)								
		Methane				Ethane				
Experiment	Sample size (g)	CH_4	CD_4	CD_3H	Other deuterated species	C_2H_6	C_2D_6	C_2D_5H	Other deuterated species	^{36}Ar§
(i) Crushing *in vacuo*†	0·670	1·3	+	+	+	0·12	+	+	+	0·11
(ii) Blank	0	+	+	+	+	+	+	+	+	+
(iii) Crushing *in vacuo* after D exchange	0·540	0·33	0·61	0·26	0·06	0·14	0·14	0·20	0·16	0·09
(iv) Crushing *in vacuo* after D exchange	0·392	0·31	0·72	0·10	0·04	0·25	0·23	0·10	0·15	0·14
(v) DCl on unsieved fines	0·201	1·8	8·4	1·1	+	*	*	*	*	0·54
(vi) DCl on unsieved fines	0·056	1·8	8·6	0·9	+	0·43	0·77	0·23	0·14	0·32
(vii) DCl on fines (30–100 mesh)	0·053	0·89	4·6	0·5	+	*	*	*	*	0·24
(viii) DCl on fines passing 200 mesh	0·054	2·4	11·1	1·5	+	*	*	*	*	0·56

+, Not detectable. * Not measured. † Trace quantities of propane were also observed. § ^{36}Ar is quoted for comparison purposes. This species is known to be trapped in the lunar fines and is virtually absent (0·34 per cent) in terrestrial argon.

Degassed D_2O (15 mg), in a small glass ampoule, was added and pulverized with the sample. The rare gases He, Ne and Ar were again present in their lunar isotopic abundances. Of the methane released, more than 95 per cent could be accounted for as CH_4, CD_4 and CD_3H; 75–80 per cent of the ethane could be accounted for as C_2H_6, C_2D_6 and C_2D_5H (Table 1).

In a third experiment aliquots of the sample were treated (16 h) with DCl (38 per cent in D_2O, isotopic purity 99·5 per cent, 1 ml.). The results of all the DCl experiments are summarized in Table 1. Fifteen per cent of the methane was released as CH_4, almost all (90 per cent) of the remainder being CD_4. Thirty per cent of the ethane was present as C_2H_6 and almost all (90 per cent) of the remainder was C_2D_6 or C_2D_5H. The quantity of methane released in experiments (vii) and (viii) (Table 1) parallels the known total carbon content of size differentiated fines[5].

Our interpretation of these data is that the greater part of the methane and ethane released by crushing or acid treatment of the fines arises from reactions brought about during the experiments. It is likely, as suggested by Ponnamperuma *et al.*, that hydrolysis of carbides, chiefly cohenite (iron, nickel carbide), is the main source of these hydrocarbons, as shown by the evolution of the CD_4, C_2D_6 and almost completely deuterated hydrocarbons. Such hydrolysis can evidently take place during the vacuum crushing process, although prolonged contact (24 h) of the fines with D_2O but without crushing released no hydrocarbons. What is more significant is that most of the remainder of the methane and ethane is completely unlabelled, proving that this fraction must have been present as such in the fines[2,3]. This is emphasized by the fact that DCl treatment of a sample of cohenite of meteoritic origin (courtesy of Dr R. Brett, Manned Spacecraft Center, Houston, Texas) produced methane comprising only CD_4 (95 per cent) and CD_3H (5 per cent).

The carbide in the lunar sample[6,7] is probably of meteoritic origin, but the trapped gaseous hydrocarbons might originally have been present in the igneous lunar rocks (primordial) or could have one or more of the following origins or formations: (i) hydrolysis of carbides *in situ* on the Moon (for example, from hydroxylic groups formed by solar wind implantation[8] of protons); (ii) thermal or radiation induced alteration of indigenous organic matter; (iii) formation from the solar wind, for example by hydrogenation of indigenous or solar wind carbon by hydrogen implanted by the solar wind. Our present data do not distinguish among these possibilities and further experiments are in progress.

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Ball Lightning

Jennison's report[1] of ball lightning inside an airliner reminds me of an observation of my own. It took place on a summer evening in either 1928 or 1929 during a severe electrical storm in the Kootenay Lake Valley, 10 miles from Nelson, BC. The event, in two parts, was seen by a group watching from a sheltered porch and a second (of which I was a member) inside a wooden cottage in a forested area by the lakeside.

Some distance from the shore the first group saw a ball of light drop from the clouds towards our side of the lake. It did not strike the mountainside but came to a stop, floating above the forest. The main road and electrical transmission line along the lakeside were here built parallel to but away from the shore, and led down into a smaller open valley stretching from the lakeside into the mountainside. The ball continued downwards, drifting above the road and the transmission line until it reached the bottom of the valley. Here it drifted to the lake shore and struck one of the piles of a small wharf jutting into the lake. It shattered the pile into long splinters and produced a large detonation.

The indoor group had not been watching the storm. While listening in the living-room to the thunder, we saw a ball of light, similar to Jennison's, emerge from the fireplace and slowly drift across the room. It appeared to pass through a curtained, closed door without making any noise above the rumble of the storm or causing any damage. A loud detonation was heard a few moments after the ball vanished.

We did not know about the first ball until the next morning. At one time the neighbour who had seen it was concerned that it might strike our cottage, but it had carried on to the pile some 200 or 300 feet away. I infer that the first ball, clearly of high energy, induced in some way the formation of the second, low energy ball that emerged from our fireplace.

Jennison's description corresponds to my memory of the indoor ball. It is remarkable that the different surroundings should produce or be accompanied by similar balls: one inside a metal aircraft moving within an electrically disturbed cloud, and the other inside a wooden cottage with a high energy ball moving overhead.

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[1] Jennison, R. C., *Nature*, **224**, 895 (1969).

JENNISON'S[1] most unusual ball lightning observation brings several questions to mind: (*a*) Was the ball observed by the crew and other passengers, and if so, what were their reactions ? (*b*) Did the crew in the pilot's cabin report any damage to or unusual behaviour of the electrical equipment ? (*c*) Where and how did the flight of the ball terminate ? (*d*) Was the airplane interior pressurized, and at what approximate altitude was the plane flying ? (*e*) Why did the observer wait more than six years to report on such an interesting phenomenon ?

I would like to add that the antenna feed is a possible access route for the lightning discharge into the airplane.

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BALL lightning is a rare phenomenen, which, because of its nature, is virtually impossible to record objectively. Carefully documented reports such as the one by Jennison[1] are therefore valuable.

A similar incident occurred in the Meteorological Office, Boscombe Down, Wiltshire, in the northern summer of 1938. There was a vivid flash with simultaneous thunder. It was assumed that the Dines pressure-tube anemometer, which passed through the roof and was supported by four heavy steel supports embedded in the floor, was struck.

Immediately after the lightning flash, a brilliant white sphere, with a diameter of about 20 cm and diffuse edges, was observed hovering near the anemometer supports. The diameter, symmetry and absence of structure agree with Jennison's description. No heat radiation was noted. The motion differed from Jennison's case. The object followed an irregular course; it travelled from one anemometer leg to the other, and then from the anemometer to a radio receiver/transmitter which was being operated at the time. The distance from the anemometer to the radio set would be about 2 m, and the height above the floor about 1·5 m. The object disappeared as it reached the radio set, perhaps 3–5 s after its appearance.

The radio operator witnessed the incident and generally agreed with the motion and other properties already described.

Although this report is thirty years old, it is believed to be reliable.

It is possible that such occurrences are attributable to physiological or psychological origin; the close agreement between different observers would not necessarily detract from this suggestion. Alternatively, photographs, claimed to be of ball lightning, have appeared in the popular press. Whatever the cause, it seems desirable to place the observation on record.

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Late Quaternary Chronology of the Nakuru–Elmenteita Basin, Kenya

IN 1967[1] I gave details of a shoreline of a former lake in the Nakuru–Elmenteita basin of Kenya at an altitude of about 6,370 feet above sea level, or about 600 feet above the present surface of Lake Nakuru. It was suggested then that, although this shoreline might still qualify to be called "Gamblian" after the formerly accepted nomenclature for the late Quaternary in East Africa[2], it is necessary to be extremely cautious in using this nomenclature, because its usage involves assumptions of dating and correlation which are not borne out by the results of recent fieldwork.

Some further evidence from the Nakuru–Elmenteita basin has been obtained in the form of a radiocarbon date for material which seems to have close links with the 600 foot shoreline. The dated sample consists of the shells of Mollusca which were found *in situ* in a matrix of pale buff diatomaceous silt. The exposure is at an altitude of 6,188–6,192 feet above sea level, or more than 400 feet above the present surface of Lake Nakuru. The material is clearly of lacustrine origin, both from the character of the silt, and from the species of Mollusca which have been identified in it.

The shells have been dated by Dr D. Thurber of Lamont Geological Observatory. The age obtained for the shells using the 5,585 half-life for radiocarbon and assuming the initial $^{14}C/^{12}C$ ratio to be atmospheric was 9,650 ± 250 years before 1950 (L–1201). Dr Thurber also analysed the $^{14}C/^{12}C$ ratio in samples of water from Lakes Nakuru and Elmenteita and has informed me that no adjustment to the above date need be made on the basis of these analyses.

Dr B. W. Sparks of the Cambridge University Department of Geography has kindly studied another sample of the shells and lists the following species: *Corbicula africana* (Krauss) (most common); *Melanoides tuberculata* (Müller) (second in abundance); and very small numbers of *Pisidium* sp., *Burnupia* sp., *Bulinus* sp., *Anisus natalensis* (Krauss) and *Biomphalaria* sp. These are all freshwater species and there were no land snails in the samples. Sparks suggested that these snails, in particular *Corbicula* and *Melanoides*, are indicative of a lake of appreciable size rather than of a small perennial or temporary body of water. It is not possible from this sample to obtain a precise indication of the depth of water or length of time implied by the deposit.

The only other record of Mollusca from the Nakuru–Elmenteita basin is that by Leakey[2]; he described shells from various localities, including Gamble's Cave. The Gamble's Cave shells included the following species: *Lymnaea elmenteitensis* ?Smith, (rare); *Bulinus* sp. (fragments); *Melanoides tuberculata* (Müll.) (very abundant); and *Corbicula africana* (Krs.). The list of Mollusca was prepared by M. Connolly and is given in Appendix D of Leakey's book[2]. The shells are described as being obtained "from the beach gravel at 510 feet above present lake level in Gamble's Cave II".

There seem to be no more recent reports of Mollusca from this basin, and I was only able to find one occurrence of shells in the diatomaceous silts. The silts containing the Mollusca at this locality are similar in appearance to many of the other outcrops of diatomaceous material which occur all over the basin. These deposits are found up to an altitude of about 6,330 feet, and many of them seem to represent sedimentation in fairly calm water below the 600-foot shoreline. It is not possible to state definitely that the silts that contain the Mollusca, which have been shown to be about 9,650 years old, are exactly contemporaneous with the 600-foot shoreline. Their relationship to certain nearby lake features, however, suggests a possible broad correlation. The Mollusca-bearing silts outcrop in a small gully on the north-east slope of the volcano Karterit (just below the "a" of the word Karterit on the map, Fig. 1 of my earlier paper[1]). Upslope from this gully are a number of other probable lake features; silts, pebble-bands, surface pebbles, and finally a possible shoreline cliff, the Karterit notch. This is an overhanging cliff, about 6 feet high, cut in the rocky slope of the volcano. It runs across the slope for several hundred yards and when conditions of light and vegetation are suitable it is clearly visible from the main Nairobi–Nakuru road. The identification of this feature as a lake shoreline is not absolutely definite; however, it does not seem to have been formed by faulting of the cone, and its altitude correlates well with that of the best preserved remnant of the 600-foot shoreline, the Menengai shoreline cliff, about 23 miles away at the north end of the Nakuru–Elmenteita basin. The altitudes are:

Base of Karterit notch:	*circa* 6,365 feet
Base of Menengai cliff:	*circa* 6,370 feet

Even if the Mollusca-bearing silts cannot definitely be linked with the 600-foot shoreline, they were clearly laid down when the lake level was over 400 feet higher than it is now. There is no evidence of a prolonged pause in the fluctuations of the lake level at any altitude between the approximately 600-foot and 400-foot levels (6,370–6,190 feet); the date of the 600-foot shoreline itself may thus be quite close to the date of the silts which lie about 180 feet below it.

Further evidence, which to some extent supports my results, has been obtained from an archaeological excavation carried out by Miss Barbara Anthony of Harvard University at Prospect Farm, which is also in the Nakuru–Elmenteita catchment area. At Prospect Farm, in an excavation well above the highest former shoreline, Miss Anthony obtained some charcoal from a horizon containing an industry known as Kenya Capsian. This charcoal was dated by Geochron Laboratory and gave a date of 10,560 ± 1,650 BP (GX–224). A Kenya Capsian industry has also been described[2] as immediately overlying the beach sand with Mollusca at Gamble's Cave. This beach sand is at an altitude of 6,344 feet and seems to be a lake sediment laid down close below the 600 foot shoreline[1]. Prospect Farm is about 7 miles from Gamble's Cave and the Prospect Farm date for the Kenya Capsian industry in the Nakuru–Elmenteita basin thus provides an indication that the Gamble's Cave beach sand may be in the vicinity of 9–11,000 years old. Further evidence of the relatively late date of the Capsian industry is provided by the bone harpoon found at Gamble's Cave and described by Oakley[3], and by numerous north African radiocarbon dates for Capsian industries.

The evidence suggests therefore that there were very high lakes in the Nakuru–Elmenteita basin as recently as 10,000 years ago. As yet, there is not sufficient information for a detailed chronology of lake level fluctuations in the basin. The evidence from this basin shows a general agreement with the sequence suggested by Richardson from his study of a core from the bed of Lake Naivasha[4]. It is hoped that further investigations in these areas will provide more information and enable firm links to be made. Until such correlations can be established between adjacent areas, which at present experience basically similar climatic conditions, it is unwise to put too much faith in wider correlations between areas of very different present day climates.

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The "Keuper" Series of the British Trias in the Northern Irish Sea and Neighbouring Areas

Considerable interest in the geology of the Irish Sea has followed from the exploration of the area for hydrocarbon deposits. We present here a brief account of the results of palynological studies of the "Keuper" Series which have been carried out at the Institute of Geological Sciences, Leeds.

The palynological examination of material from the Institute of Geological Sciences (IGS) boreholes in Cheshire, the Irish Sea and Northern Ireland, and from Imperial Chemical Industries Ltd boreholes in Cheshire, has yielded information relevant to the study of the northern part of the Irish Sea.

In Cheshire the "Keuper" sequence (shown diagrammatically in Fig. 1) has yielded miospore assemblages which give the age data shown in Table 1.

Table 1. AGE OF CHESHIRE "KEUPER" SEQUENCE

Location	Borehole	Age
360 feet above base of the Middle "Keuper" Marl	Wilkesley Borehole (IGS)	Ladinian
Basal part of Middle "Keuper" Marl	Wardle Borehole (IGS)	Ladinian
2 feet above base of the Middle "Keuper" Marl	Holford (ICI) borehole	Anisian
Approx. 80 feet above the base of the "Lower" "Keuper" Saliferous beds	Holford (ICI) borehole	Anisian
122 feet below the top of the Lower "Keuper" Marl	Wilkesley Borehole (IGS)	?Anisian
58 feet above the base of the Lower "Keuper" Marl	Wilkesley Borehole (IGS)	Upper Scythian —Anisian
Waterstones	Houndslough Borehole (IGS)	Upper Scythian
"Keuper" Sandstone[1]	Alderley Edge (outcrop)	Upper Scythian

In the Irish Sea, a core of "Keuper" Marl facies from the IGS Continental Shelf Unit 69/2 Borehole, situated 53° 40′ 30″ N, 04° 03′ 09″ W, about 37 miles west of the mouth of the River Ribble[2], has yielded miospores suggestive of an upper Scythian age.

In Northern Ireland, the IGS Langford Lodge Borehole on the eastern side of Lough Neagh proved to have a "Keuper" sequence which consisted entirely of "Keuper" Marl facies. Miospore assemblages from this section have shown that the "Keuper" Marl includes deposits, ranging in age from upper Scythian at the base to ?Norian at the top[3]. The lower part of the Langford Lodge sequence is therefore the time correlative of entirely different facies, the "Keuper" Sandstone and Waterstones, in Cheshire (see Fig. 1). It seems therefore that the "Keuper" sequence in the northern Irish Sea area could consist largely of "Keuper" Marl facies, an interpretation which is supported by evidence from the Continental Shelf Unit borehole.

The Lower (Anisian) Saliferous Beds of Cheshire are overlain by argillaceous deposits (the Middle "Keuper"

Marl) which are now known to be partly of Ladinian age, and which are succeeded by the upper saliferous beds for which no direct evidence of age is available. In view of the age of the Middle "Keuper" Marl, however, and the fact that a similar thick halite has yielded Carnian miospores[1], the upper halite in Cheshire may be of Carnian age. It is significant that the halite units are restricted to the "Keuper" Marl facies, which is thought to have accumulated in a hypersaline environment. The presence of microplankton (*Micrhystridium*, *Veryhachium* and *Tasmanites*) in palynological residues from the Lower and Middle "Keuper" Marls of Cheshire and the basal "Keuper" Marl at Maer, Staffordshire, indicates that the water in which these deposits accumulated was derived from a marine source.

The Waterstones and "Keuper" Sandstone which underlie the "Keuper" Marl in Cheshire and much of the Central Midlands represent, respectively, the deposits of a littoral or estuarine marine or semi-marine and a fresh-water fluvial deltaic environment[1]. Palynological data from Worcestershire[1,4], which was up-river from Cheshire during the deposition of the "Keuper" Sandstone but into which the hypersaline environment gradually transgressed during the later Trias[1], indicate that the "Keuper" Sandstone and Waterstones of Worcestershire are the time-correlatives of the "Keuper" Sandstone, Waterstones, Lower "Keuper" Marl, Lower Saliferous Beds and probably part of the Middle "Keuper" Marl of Cheshire (Fig. 1). A knowledge of the distribution and the ages of the main "Keuper" facies in different areas makes it possible to suggest where halite may have formed at a particular time. It is clear, for example, that a halite representative of the Lower Saliferous Beds of Cheshire will not be found in Worcestershire because, during the Anisian, that area was occupied by a freshwater fluvial environment. Later, however, a hypersaline environment pervaded both Cheshire and the Central Midlands, and an important halite of proved or probable Carnian age is found in Cheshire (the Upper Saliferous Beds), and in Staffordshire and Worcestershire. It therefore seems that halites of approximately the same age as the Anisian and Carnian halites of the North Sea Basin[5,6] occur in the Midlands where a "Keuper" Marl facies of the appropriate age occurs. The Cheshire Basin sequence may also include an upper Scythian halite because traces of halite occur within the basal 350 feet of the Lower "Keuper" Marl[7]. Similar reasoning, applied to the situation in the northern Irish Sea and Northern Ireland where the "Keuper" Marl facies includes deposits of upper Scythian age, suggests that three halites corresponding to the upper Scythian, Anisian and Carnian halites of the North Sea Basin may also occur there (Fig. 1).

The existence of halite deposits in the "Keuper" Marl of the Isle of Man[8], Walney Island (Furness)[9] and at Preesall in west Lancashire[10] has been known for some time. In view of the proximity of the Isle of Man halite and the lower of the two Walney Island halites to the base of the "Keuper" Marl facies, it is possible that these may be of upper Scythian age. In the absence of material from the evaporites at these localities, however, their age must remain conjectural. Work currently being carried out by the IGS in west Lancashire is planned to elucidate the halite sequence in that area. In Northern Ireland, the Langford Lodge Borehole, on which palynological work has been carried out, was chipped and there is no record of the existence of any halite deposits in that section. In the IGS Larne Borehole[11], however, on which palynological work remains to be done, a section of over 3,000 feet (923 m) of "Keuper" Marl facies included three distinct halite units. These halites may prove to be

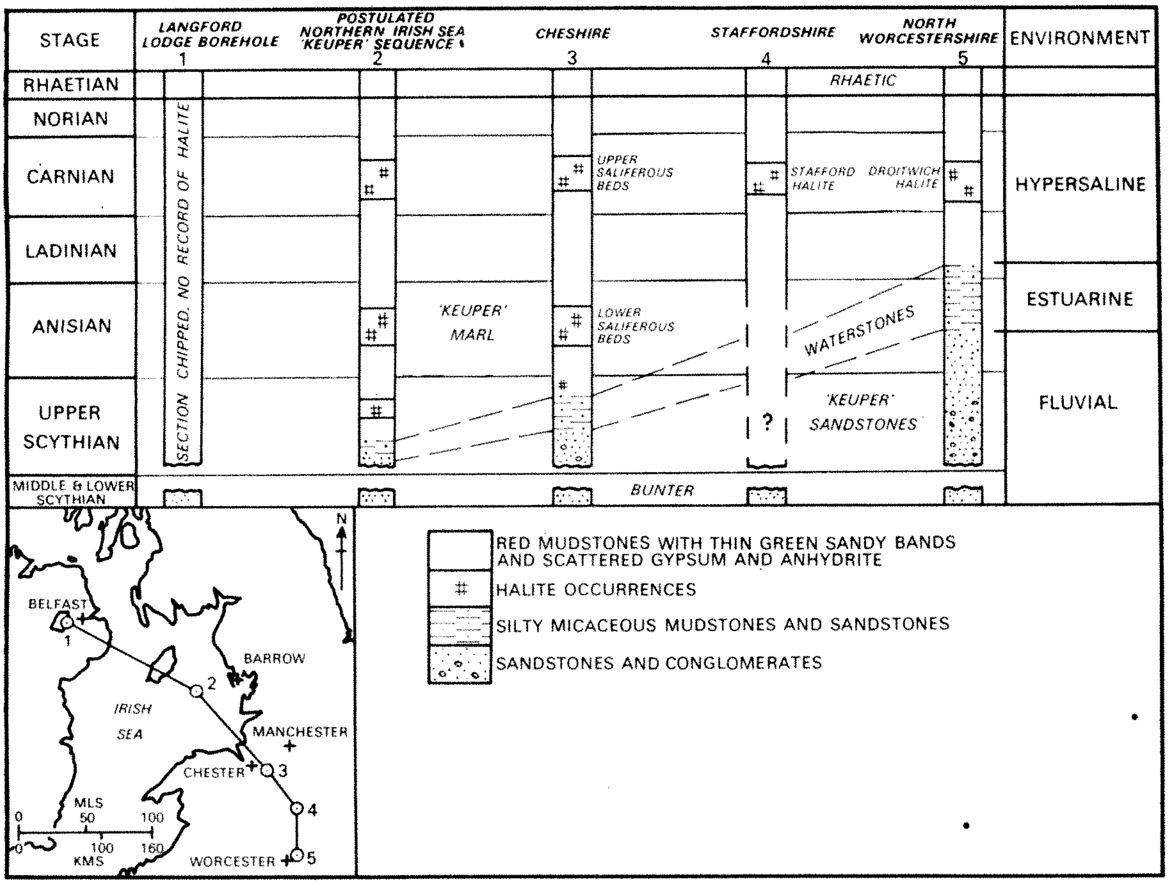

Fig. 1. The time-facies relationships (diagrammatic) in the "Keuper" series of the West Midlands, Cheshire and the northern Irish Sea area.

correlatives of the major halite units of the North Sea sequence.

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Transform Faulting along the De Geer Line

DE GEER[1] in 1926 first referred to the existence of a physiographic lineament following the western continental slope of the Barents Shelf from Norway to Svalbard, then running along the north-eastern continental slope of Greenland and Canada. Subsequent authors[2,3] suggested that extensive dextral strike slip movements had occurred along this line during the continental drift of Greenland and North America away from Eurasia.

Harland[4] has noted that the implied reconstruction is consistent with similarities between the Mesozoic and Tertiary stratigraphy in Spitsbergen and the Canadian Arctic.

Conversely, sinistral transcurrent faulting has been postulated[5] along the line of the East Greenland and North Eurasian continental margins. This hypothesis is refuted by the evidence presented here and by the distribution of seismicity[6,7] and magnetic anomalies[8] in the Arctic Ocean.

Recent bathymetric surveys have revealed the general morphology of the Norwegian and Greenland Seas[7,8] and of the Arctic Ocean[9] (Fig. 1). The seismic activity in the area is largely restricted to a linear zone, part of which runs along the Spitsbergen Fracture Zone[7], parallel to the De Geer Line.

Four earthquakes along the Spitsbergen Fracture Zone, occurring since the establishment in 1962 of the USCGS World Wide Standardized Seismograph Network, have had body wave magnitudes greater than 5·0. These are listed in Table 1.

A study of the first arrivals of P waves from these resulted in focal mechanism solutions for events 2 and 3 (Fig. 2). Solutions were not obtained for events 1 and 4, whose seismograms were unreadable because of a gradual onset (event 1) and a high noise level following a large event elsewhere (event 4). The focal mechanisms of events 2 and 3 both indicate two steeply dipping, orthogonal nodal planes which strike about 310° and 40°. Of each pair, that striking about north-west was chosen as the fault plane, for the following reasons: (1) the line of earthquake epicentres follows a north-west trend; (2) this trend is closely parallel to the bathymetric feature of the Spitsbergen Fracture Zone; (3) continental structures of Mesozoic and Caenozoic age in Spitsbergen show similar trends. The Spitsbergen Fracture Zone is thus confirmed to be an active transform fault[12] with dextral strike slip motion, separating the spreading axis of the Arctic Ocean from that of the North Greenland Sea.

Table 1. FOUR EARTHQUAKES ALONG THE SPITSBERGEN FRACTURE ZONE

Event No. (this paper)	USCGS event No.	Date	Origin time GMT	Epicentre Lat.	Epicentre Long.	Body wave magnitude
1	4,439	Nov. 27, 1966	20–13–1·5	78·50 N	6·40 E	5·6
2	3,962	Oct. 18, 1967	1–11–44·8	79·80 N	2·40 E	5·7
3	4,415	Nov. 23, 1967	13–42–1·6	80·20 N	1·00 W	5·8
4	1,256	Apr. 7, 1968	5–16–24·9	81·50 N	3·90 W	5·3

According to the theory of plate tectonics[13], it is possible with the present and other data[7,14] to determine the pole of rotation of Greenland and North America with respect to Europe. Great circles were drawn through the epicentres perpendicular to the slip vector for the strike slip displacement events on the Spitsbergen Fracture Zone, and north of Iceland[7], and for a normal displacement event in the Laptev Sea[7]. These great circles for the three strike slip faults are all very close together, and intersections between them lack control along their arcs. The normal faulting solution from the Laptev Sea does give some control, however, although it must be assumed that of the two nodal planes obtained by Sykes, that striking N 338° E is the fault plane. The intersections thus obtained are shown by closed circles in Fig. 3. They have a mean position of 124° E ± 5°, 66° N ± 5°. The angular velocity of the rotation about this pole, based on magnetic data from the Reykjanes Ridge[13], is $3 \cdot 7 \times 10^{-7}$ degrees yr^{-1}.

This may be the best position of the instantaneous rotation pole of Greenland and N. America, with respect to Europe, that can be determined with the data now available, but it is emphasized that further data should yield a more accurate position. It is perhaps worth noting that this pole seems to be further south-east than the poles obtained by Bullard, Everett and Smith[15] and Le Pichon[16], probably because these poles pertain to the total movements of the two plates, rather than to the (geologically) instantaneous pole.

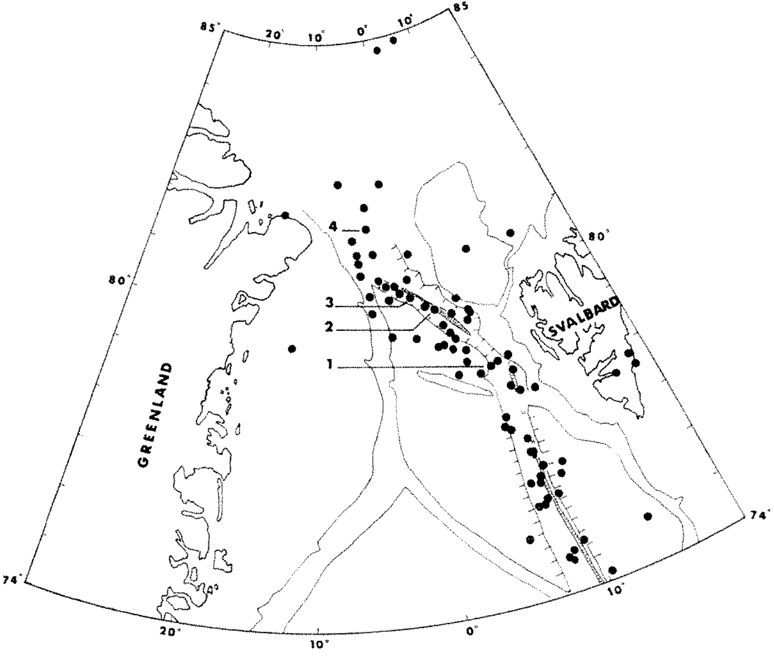
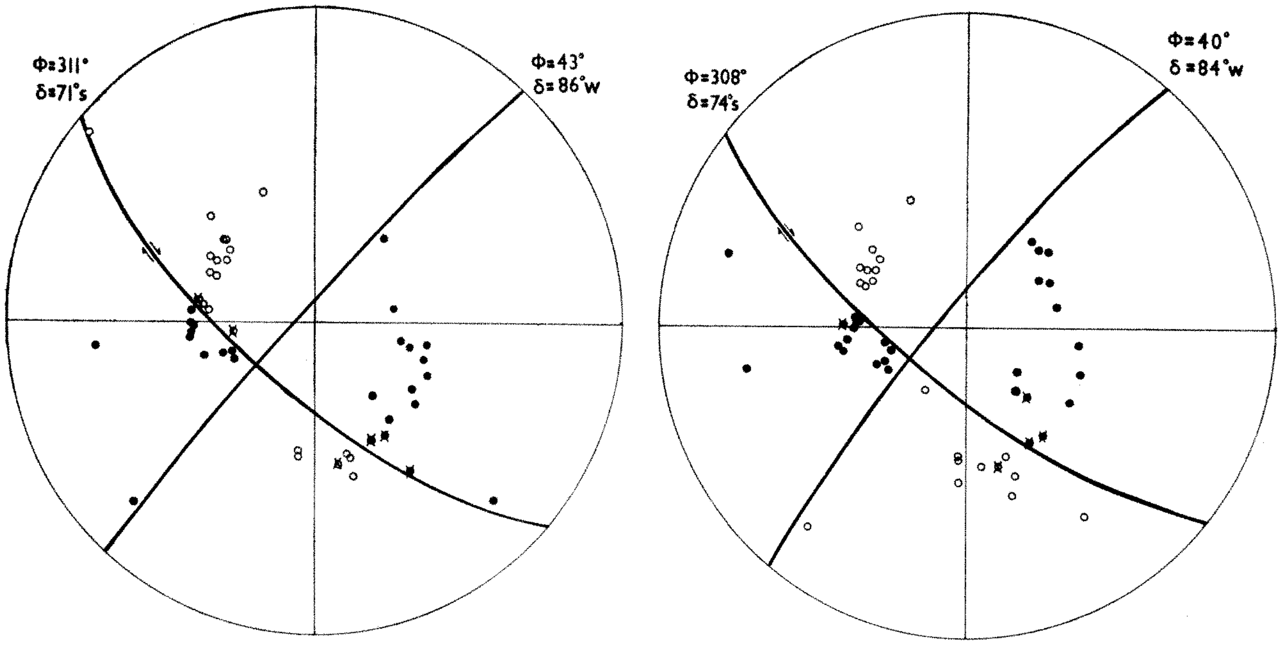


Fig. 1. Outline of bathymetry of the Norwegian–Greenland Sea between Greenland and Svalbard, after Johnson and Eckhoff[9]. Solid circles are earthquake epicentres after Sykes[7], and subsequent USCGS lists. Numbers indicate locations of events listed in Table 1, which lie along the Spitsbergen Fracture Zone.



Oct. 18, 1967

Nov. 23, 1967

Fig. 2. Focal mechanism solutions for events 2 and 3 on the Spitsbergen Fracture Zone. Equal area projection of the lower hemisphere of the radiation field. Solid circles indicate compressional first arrivals, open circles dilatational ones. Crosses mark stations near nodal planes. Arrows indicate sense of displacement on plane chosen as the fault plane.

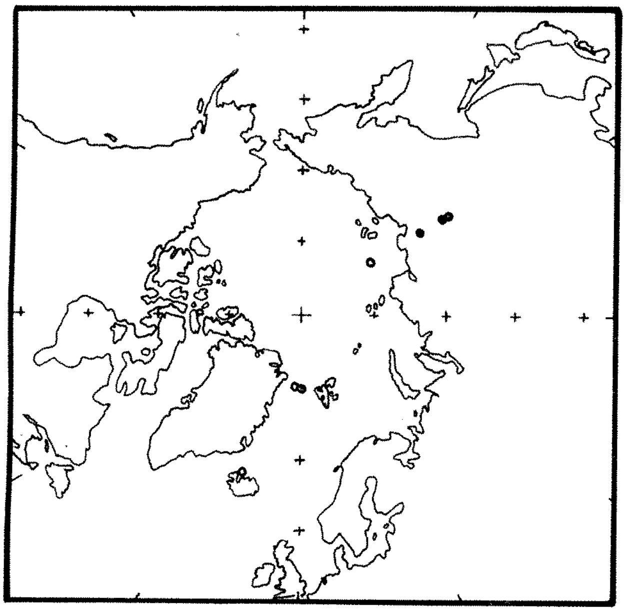


Fig. 3. Equal area projection of northern continents. Open circles mark location of epicentres used to determine instantaneous rotation pole of European plate relative to N. America–Greenland plate. Solid circles are intersections of normals to slip vectors—see text.

We thank D. P. McKenzie for his help.

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Orientation of 3-D Graphite in Carbon Fibres

CARBON fibres have been produced that have high strength and stiffness and a polycrystalline structure consisting of carbon crystallites oriented in the direction of the fibre axis[1,2]. Some evidence for recrystallization has been reported for carbon fibres heat treated in a nickel matrix[3,4] and in boron[5]. Here we consider the effect of recrystallization on the preferred orientation of carbon fibres during an improvement in order of the carbon atoms.

As part of a cooperative programme directed towards an understanding of the fundamental structure of carbon fibres, heat treatments have been performed (at the Weapons Research Establishment, Adelaide) over the temperature range 1,600°–3,000° C for periods of 10–20 min, using a dynamic atmosphere of argon and a carbon block container. A heterogeneous surface deposit was produced in suitable conditions during heat treatment as a result of impurities contained in the carbon block. Regions of the fibres on which the surface deposit occurred could be separated magnetically from clean regions and the contamination studied directly.

Fig. 1 is a scanning electron micrograph of a bundle of fibres, and shows the typically globular nature of the deposit. X-ray analysis shows that the surface deposit is a ^{14}Fe per cent Si solid solution, and is supported by spectrographic analysis. Estimates of the effective size and orientation of carbon crystallites in regions selected by direct observation were then made by X-ray diffraction and electron microscopy.

The degree of preferred orientation of the carbon crystallites aligned along the fibre axis was estimated from a measure of the half widths of the (002) diffraction arc. The effective size of the crystallites normal and parallel to the basal planes was estimated from the line broadening of the (002) and (100) diffraction arcs respectively.

The effect of surface contamination on the graphitization of a carbon fibre at 2,500° C is shown in Fig. 2. The X-ray pattern (Fig. 2*a*) of a clean area is typical of high-modulus carbon fibre[6] with the basal planes highly oriented along the fibre axis and a crystallite size of approximately 50 Å in both the *a* and *c* directions. Fig. 2*b* is typical of fibres with fine surface deposits—the (101) reflexion indicates the presence of three-dimensional-ordered (3-D) graphite which has a measured interlayer spacing of 3·35 Å. The average crystallite size of the 3-D graphite is too large to be measured by line broadening. The marked change in the (002) diffraction line from arcs to a uniform ring illustrates the reduction in basal plane alignment along the fibre axis. This change is considerable, for Fig. 2*a* shows (002) arcs with a distribution half width of 30°, whereas in Fig. 2*b* the full ring is present with arcs of half width 54° superimposed.

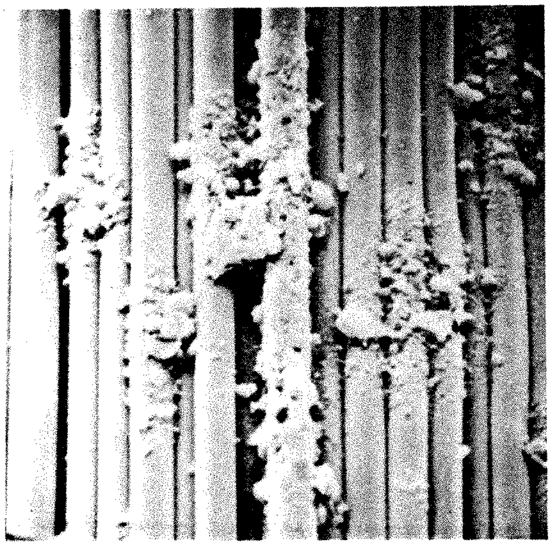

Fig. 1. Scanning electron micrograph showing Fe-14 per cent Si surface deposits on carbon fibre heat treated to 2,500° C (by courtesy of V. Silva, Defence Standards Laboratories, Melbourne).

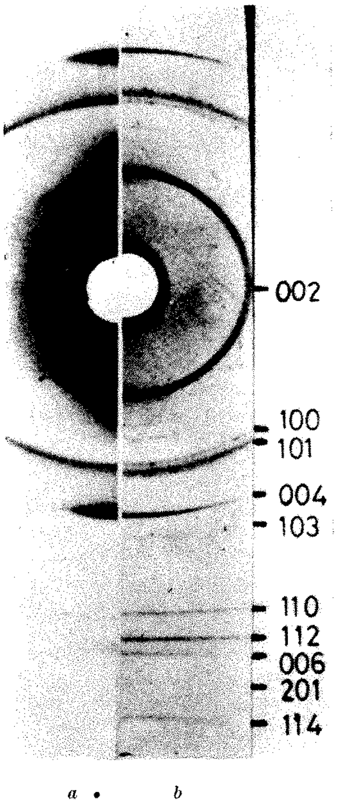

Fig. 2. X-ray transmission pattern of carbon fibre heat treated to 2,500° C. *a*, Area free from surface deposit, showing normal preferred orientation; *b*, result of surface deposit showing 3-D order and reduced preferred orientation.

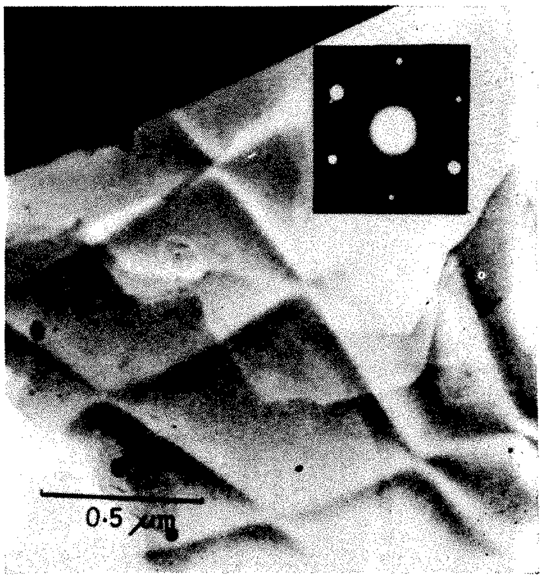

Fig. 3. Electron microscope picture of single crystal graphite in contaminated carbon fibre. The network of dislocations shows contracted nodes.

Using a flame polishing technique, selected areas of the fibre similar to those corresponding to Fig. 2*b* were examined in the electron microscope. Fig. 3 confirms the presence of 3-D graphite, and shows a network of basal dislocations identical to that observed in single crystal graphite. Graphite platelets are observed in these regions in completely random orientation, bearing little relation to the original microstructure.

Preliminary mechanical tests suggest that lengths coated with the surface deposit have lower fracture strengths than clean fibre lengths. This implies that the presence of single crystal graphite platelets oriented perpendicular to the fibre axis may be important in reducing the fracture stress of commercially produced high-modulus fibres[2].

The role of the ^{14}Fe per cent Si deposit in catalysing the growth of three-dimensional graphite platelets is similar to the formation of graphite Kish crystals on the surface of Fe-Si casting melts[7]. The nucleation conditions, the tendency towards a random growth of the graphite platelets, and the effect on fibre fracture stress are being further investigated.

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Flow at the Junction of Two Pipes: the Shape of the Separation Surface

THE subject of flow in branched pipes is largely unexplored. Some work has been carried out by Bugliarello and Hsiao[1,2] and others (see ref. 3, and references therein), principally with an eye to applications in the microcirculatory system. The problem of "what fluid goes where" is particularly vital in the case where the destination of suspended particles is in question. This is naturally one of the major questions of circulatory physiology.

To provide a basis for the study of flow at a junction of two pipes, we have studied several particularly simple cases. We have avoided the sharp-edged junctions of Bugliarello and Hsiao[1,2], for we agree with the criticism of these expressed by Neumaster and Krovetz[3]. Moreover, in order to elucidate basic phenomena we have worked entirely with a Newtonian fluid (water)—previous studies have used blood. The basic situation is as follows.

A cylindrical pipe of diameter d ("the branch") leads off from the side of another of diameter D ("the tube"), where $d < D$. Fluid is forced under pressure down the tube; some is drawn off from the branch, and the rest will continue down the tube. A surface may be envisaged dividing these two portions of fluid. This, following Bugliarello and Hsiao[1], we term the "separation surface". We report here on a series of experiments to determine the shape of this surface far upstream from the junction. Our experiments cover only the case where the two pipes meet at right angles. We believe this to be the first experimental determination of a separation surface.

In our apparatus, water flowed under gravity down a 161·0 cm length of 4·76 cm diameter (internal) brass tubing, which was fitted flush into a bored 'Perspex' block of matching internal diameter. Replaceable sections of this block allowed for varying ratios d/D to be investigated. The values we chose were: 0·053, 0·106, 0·159, 0·212. The edges of the junction were bevelled to eliminate sharp corners.

Below the junction the water flowed through peristaltic pumps fitted to both tube and branch. Each pump was stabilized with a Helmholtz resonator so that the resulting flow was steady to within 8 per cent in the branch and 3 per cent in the tube. The water was then returned to the top of the tube.

According to Schlichting's inlet length theory[4], the length of tube made feasible Reynolds numbers (Re) of up to 200 without losing fully developed Poiseuille flow. We were able to achieve Reynolds numbers as low as 30 before the times involved in the experiment became impracticably great. This covers a range of biologically applicable Reynolds numbers but leaves some of the area relevant to the microcirculation unexplored. It will be noted, however, that the results seem to be largely independent of Reynolds number.

The ratio β between the volume rates at which fluid was extracted from the branch and from the tube could also be varied. We did not, however, investigate flow profiles other than parabolic, although it would be of interest to see if the results described here apply also to plug flows.

The flow was made visible by the almost neutrally buoyant dye "Edicol Supra" Blue VRS, leaked in through a hypodermic syringe 3·55 cm upstream from the junction. The syringe could be moved backwards and forwards with complete freedom by means of a micrometer screw. There were nine small holes drilled into the 'Perspex', just large enough to admit the syringe. Those not in use were blocked up with small plugs. One of these holes was drilled along a diameter of the tube on the side directly opposite the branch and parallel with the branch. The other eight were parallel to the first and evenly spaced across half the diameter of the tube on the side opposite the branch. The shape found for the separation surface was completed by symmetry.

Readings were made by moving the syringe in from outside the separation surface, through the surface and back again. Three sets of readings were taken. The position of the dye stream we used was that at which the dye divided equally between branch and tube.

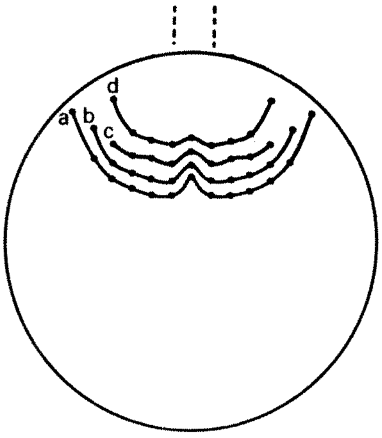

Fig. 1

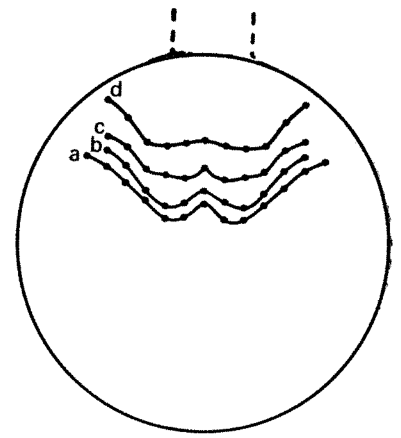

Fig. 2

Fig. 1. Separation surfaces for Re = 123·7, $d/D = 0{\cdot}106$, $\beta = 0{\cdot}107$ (*a*), 0·083 (*b*), 0·061 (*c*), 0·041 (*d*).

Fig. 2. Separation surfaces for Re = 123·7, $d/D = 0{\cdot}212$, $\beta = 0{\cdot}107$ (*a*), 0·083 (*b*), 0·061 (*c*), 0·041 (*d*)

The results are shown in Figs. 1 and 2. No points are shown close to the walls because flow was so slow here that repeatable results could not be obtained. The two cases Re = 123·7; $d/D = 0{\cdot}106$, 0·212 are shown in Figs. 1 and 2 respectively for values of $\beta = 0{\cdot}041$, 0·061, 0·083, 0·107. Also investigated were $d/D = 0{\cdot}053$, 0·159.

Within the stipulated range of values for Re (30 to 200) and for constant β, d/D, no change in the separation surface could be detected. When d/D is increased for constant Re, β, however, more fluid is extracted from regions near to the wall; the profile thus appears flattened and is situated nearer to the branch wall (compare Figs. 1 and 2).

It will be noticed that the shape found for the separation surface far upstream is similar to one of the various shapes postulated by Bugliarello and Hsiao[2], except that the non-repeatability of results near the wall means that we cannot consistently define a separation surface in this area and expect the result to be meaningful in practice. The prime cause of the non-repeatability was the residual unsteadiness of flow in both tube and branch.

Work is proceeding on the theoretical modelling of the phenomenon. Any successful account will have to explain in particular the marked dip in the curves close to the line of symmetry and the constancy of this feature over a wide range of experimental conditions.

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BIOLOGICAL SCIENCES

Re-examination of a Supposed Case of Specific Cell Adhesion

SPECIFIC adhesion of sponge cells, such that only the cells of a single species will adhere together, has been claimed by many workers[1-3]. Indeed, the particular specific behaviour which has been reported[3] for mixtures of cells from pairs taken from *Haliclona occulata*, *Halichondria panicea*, *Microciona prolifera* and *Clione celata* has been regarded as one of the most remarkable examples of specific adhesion or cell recognition. Sara[4-6] has tested combinations of other species of marine sponge for specific adhesion without success. In all work on sponge cell aggregation, the recognition of the species type of the cells in the aggregates has depended on subjective observation of the colour of the aggregate. I find this criterion defective, for it is extremely easy to fail to observe a small proportion of cells of one colour and species type scattered in a mass of cells of a different colour and species type. Furthermore, only a proportion of the cells of highly coloured sponges may carry the pigment distinctive of a species, so that it is uncertain what happens to the unpigmented cells.

Another difficulty lies in the possibility that the claimed separation of cells in a mixture of two species types is the result of the aggregation of one species type preceding that of the other, so that aggregates tend to contain cells of one species type alone—a form of behaviour which gives the same result as specific adhesion. I have recently introduced an experimental test for specific adhesion, which does not require the identification of the species type of the cells[7]. It depends on the measurement of the collision efficiency of aggregating cells in a suspension culture. Collisions are produced by stirring or shaking the suspension. The collision efficiency is the probability that two cells, or a cell and an aggregate, or two aggregates adhere on collision. The collision efficiency (stability ratio) is a function of the adhesiveness of the cells: the greater the probability of adhesion, the greater the adhesiveness. (The relationship has been treated in detail in ref. 8.) The basis of the technique can perhaps be best appreciated from an example. Suppose first that two different cell types are of identical adhesiveness and thus collision efficiency, and are non-specific in their adhesion. The adhesiveness and collision efficiency of any mixture of the two cell types will be the same as that for either type alone. But if there is specificity of adhesion, then—because collisions take place between cells of unlike type which will be totally ineffective in producing adhesions—the measured collision efficiency is smaller than that for either cell type alone. The expected collision efficiency in the case of a mixture of two types of cell, in proportions n_1 and n_2, is $\alpha_1 n_1 + \alpha_2 n_2$ for non-specificity of adhesion and $\alpha_1 n_1^2 + \alpha_2 n_2^2$ for specific adhesion, where α_1 and α_2 are the collision efficiencies of cell types 1 and 2. (The full theory is given in ref. 9.) The collision efficiency of cell suspensions is measured by aggregating the suspensions under a known shear rate in a Couette viscometer and determining the total particle concentration at various intervals by haemocytometry or Coulter counting[10]. Application of the treatment of particle collisions in a laminar shear flow according to Swift and Friedlander[11] allows calculation of the collision efficiency. Because this technique avoids the problems of identification of individual cells inherent in other methods and allows a quantitative measure of any specificity that may be found, I feel that it is appropriate for the re-examination of the nature of the adhesiveness in the sponges Humphreys used.

Because *Microciona prolifera* seems to be unavailable in the United Kingdom, these experiments were confined to *Haliclona occulata* and *Halichondria panicea*. Sponges were collected live at Linne Mhurich, Argyll (National Grid reference NR 724827), and dispersed into cell suspensions using the calcium chelating agent EDTA[7]. This technique is similar to that used by Humphreys, the main difference being that the cells are exposed to media free of Ca^{2+} and Mg^{2+} for a shorter time. The cell suspensions from each species were diluted extensively with artificial seawater. The particle (cell) concentration in these nearly monodisperse suspensions was measured by haemocytometry. The collision efficiencies of both the two pure cell types alone and the mixtures of various proportions were then measured using a Couette viscometer to obtain a known shear rate.

The results are shown in Fig. 1. The points are fitted by the linear regression $y = 0{\cdot}137x + 14{\cdot}1$; $s_b = 0{\cdot}0196$, where y is the collision efficiency and x is the proportion of the more adhesive cell type. The relationship which would be obtained for 100 per cent specific adhesion is shown by the dashed line. The greatest difference between these plots is at $x = 50$ per cent. Applying tests given by Snedecor and Cochran[12] for the confidence limits of a

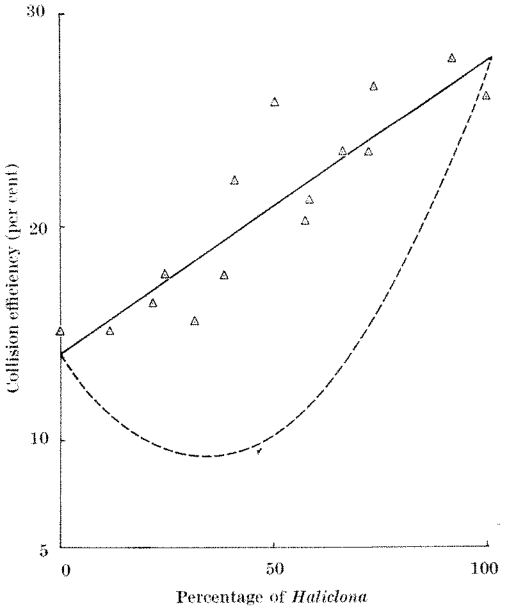

Fig. 1. Relationship between collision efficiencies (adhesiveness) and the proportion of *Haliclona occulata* cells in mixed suspensions of this species and *Halichondria panicea*. The full line shows the linear regression fitted to the plotted experimental points; the dashed line, that expected if a specific adhesion occurs.

regression, the 95 per cent and 99 per cent confidence limits are $\pm 1{\cdot}10$ and $\pm 1{\cdot}53$ for $x = 50$. The probability of 11 per cent of the adhesions being specific is $P < 0{\cdot}05$; for 15 per cent it is $P < 0{\cdot}01$. I therefore conclude that there is evidence against a general specificity of cell adhesion for these two species of sponge, though it cannot be excluded that a small proportion of cells might show specific adhesion. This, however, applies only to the behaviour of the cells during the first 40 min of re-aggregation and although Humphreys claimed that the specificity is manifest in this pair of species from the start of aggregation it is possible that the specificity only develops at a later time. Roth[13], for example, has suggested that specificity of adhesion only develops in mixtures of chick neural retina and liver cells after some hours of incubation.

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Inhibition of Cell Aggregation by Antibodies directed against Actomyosin

IT has been proposed that cell adhesion involves the participation of actomyosin-like protein located in the surface[1]. The theory was based mainly on the similarity between actomyosin proteins in muscle[2-5] and non-muscle cells[6-8], a comparison of the properties of actomyosin protein with the effects of calcium and of nucleoside phosphates[9-11] on cell aggregation, and evidence indicating the presence of actomyosin-like protein at the surface of liver cells[12-15]. Hypothetical models have been suggested for the involvement of contractile actomyosin in intermolecular bonding between adjacent cells[16-18].

According to the actomyosin theory of cell adhesion, the adsorption at the cell periphery of antibodies directed specifically against the myosin–ATPase portion of these proteins should hinder their activity and reduce cell adhesiveness.

There is good evidence using fluorescent antibody techniques that the antigenic character of striated muscle myosin is different from that of the surface myosin-like protein of liver cells[15,19] and of thrombosthenin. Antibodies against human uterus actomyosin (UAM) reacted with the smooth muscle actomyosin of vascular[20] and intestinal origin and, in addition, with fibroblasts of connective tissue and thrombocytes (platelets), but not with skeletal or cardiac muscle, nor with the $(\text{Na}^+,\text{K}^+,\text{Mg}^{2+})$ dependent ATPase from human erythrocyte ghosts[21]. Antibodies against actomyosin from human striated pectoral muscle (PAM) reacted exclusively with skeletal and cardiac actomyosins[20]. Preimmunization serum did not react in the muscle, cell and ATPase systems already mentioned. Antibodies against actomyosin from the smooth muscle of human uterus (anti-UAM) and those against the actomyosin of human pectoral muscle (anti-PAM) blocked the activity of the Ca^{2+}-dependent myosin ATPase[22]. They also reacted—as demonstrated by immunofluorescent staining—with smooth and striated muscle fibres of bovine, rabbit, rat and chicken origin. Thus although type specific, the anti-UAM and anti-PAM were not species specific.

The fact that the anti-UAM antibodies specifically blocked the activity of the Ca^{2+}-dependent actomyosin ATPase and reacted with fibroblasts of connective tissue and thrombocytes, irrespective of species origin, suggested that the effect of anti-UAM could yield information on the aggregation of liver and muscle cells from chick embryos. We now report qualitatively reproducible results from aggregation experiments with anti-UAM and anti-PAM and discuss their bearing on the actomyosin theory of cell adhesion.

Highly purified actomyosins were prepared from fresh human surgical specimens of uterine[23] and pectoral[24] muscle. Antisera against UAM and PAM were obtained in rabbits by repeated intravenous injections of these preparations[25], and tested for specificity[20]. The skeletal muscle and liver tissue of 9 day old chick embryos were dissociated into separate cells by treatment with 0·25 per cent trypsin (Difco, 1 : 250) in Hanks's balanced salt solution for 10 min at 37° C (ref. 26).

Cell reaggregation was quantitatively estimated utilizing the gyratory shaker[27] and turbidimetric[28] methods; the cells were suspended and rotated in Eagle's minimal essential medium containing 10 per cent of the test serum. The muscle and liver cells were rotated in the gyratory shaker for 24 h, progress in aggregation being estimated by single cell counts and by an examination of samples taken from the test preparations. Changes in the absorbance of cell suspensions, which can be correlated with progress in aggregation, were assessed by the turbidimetric method[11,26,28].

In the presence of calf serum, pre-immunization serum (control preparations), or anti-PAM, both the liver and muscle cells after 24 h in the gyratory shaker had formed large compact aggregates. Those in the anti-UAM preparations were conspicuously smaller and less compact, and, in addition, about 20 per cent of the total number of cells (compared with about 5 per cent in the anti-PAM and control preparations) remained free. Cell viability tests using lissamine green stain[25] showed that the number of non-viable cells in the anti-UAM preparations was the same as in the anti-PAM and control preparations at the end of the test period, so that anti-UAM was not producing its inhibitory effect by impairing cell viability.

When preparations of muscle cells were rotated in the presence of anti-UAM, the decrease in absorbance was markedly less than for preparations of the same cells containing anti-PAM or control sera (Fig. 1). This is consistent with the aggregates formed in the anti-UAM preparations being considerably smaller than those in the anti-PAM and control preparations.

The specific reactive properties of the anti-UAM strongly point to the aggregation-inhibitory effect of the anti-UAM being an expression of its reaction with actomyosin-like protein located at the surfaces of the embryonic chick muscle and liver cells. Thus the antigenic property of the surface localized actomyosin-like protein of these cells must be similar, and, as indicated by the failure of the anti-PAM to produce the inhibitory effect, different from that of the myofibrillar actomyosin of the muscle cells. This is understandable, in terms of the difference between the antigenic property of striated muscle actomyosin and the surface localized actomyosin-like protein of liver cells[15]. The marked cell aggregation–inhibitory effect of the anti-UAM may therefore be interpreted as directly supporting the theory that cell adhesion involves the participation of surface localized actomyosin-like protein[1].

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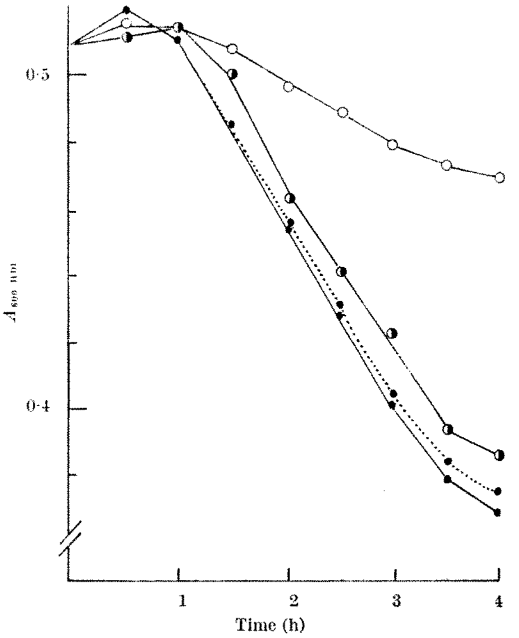

Fig. 1. The effects of anti-UAM, reactive with fibroblasts of connective tissue and thrombocytes, and of anti-PAM, on the absorbance of a rotated suspension of crude trypsin-dissociated cells from the muscle tissue of 9 day old chick embryos. Pre-immunization serum and calf serum were used as controls. ○—○, Anti-UAM (coincided with the production of small loosely formed aggregates with 20 per cent of the cells remaining free); ◑—◑, anti-PAM (well-formed aggregates comparable with those in control preparations); ●- - -●, pre-immunization serum and ●—●, calf serum (in each, production of large compact aggregates with only 5 per cent of the cells remaining free).

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Homing of Lymph-borne Immunoblasts to the Gut

Between 3 and 6 days after antigenic stimulation, lymph nodes discharge large numbers of basophilic blast cells (immunoblasts) into the efferent lymph which carries them to the blood[1-3]. These cells contain many polyribosomes but little endoplasmic reticulum; nevertheless, they produce specific antibody[1,4,5] and are specifically cytotoxic *in vitro*[6]. Unlike small lymphocytes they incorporate DNA precursors with avidity and some of them transform into true plasma cells *in vivo*[7]. Immunoblasts produced by the caudal lymph nodes of rats can be obtained by collecting the thoracic duct lymph 3–6 days after antigenic stimulation of the hindquarter regions[8,9].

Because immunoblasts are released in large numbers before antibodies appear in the blood serum, it has been suggested that their most important function is to extravasate into the tissues and so provide, by local synthesis, the antibody that the blood is unable to supply[10]. This proposition was tested by collecting immunoblasts from rat thoracic duct lymph, labelling them *in vitro* with either ^{3}H-thymidine or ^{125}iodo-2-deoxyuridine and then injecting them intravenously into syngeneic recipients. The distribution of the labelled cells was then determined by autoradiography and scintillation counting.

Pure-line, SPF, male hooded rats, weighing about 220 g, were used. Donor rats were immunized by subcutaneous injections of an antigen given into the hind foot-pads, the glutei, the inner surface of the thighs and the flanks. The antigens used were *Brucella abortus* (Wellcome), BCG (Glaxo), sheep red cells or irradiated syngeneic sarcomata[8]. Three days later the thoracic ducts of four to six donor animals were cannulated and the lymph was collected in the usual manner[8]. Lymph collected over 8–12 h periods was pooled and the number of lymphocytes and the percentage of immunoblasts were determined[8]. Each pooled collection was incubated for 1 h at 38° C with 1 μCi/ml. of either ^{3}H-thymidine or ^{125}I-iododeoxyuridine (Radiochemical Centre, Amersham). The labelled cells were washed and finally resuspended in 2 ml. of medium 'TC199' and injected immediately into the tail vein of a recipient rat; a measured portion of the injection was retained for autoradiography and/or radio assay. Usually about 5×10^{8} total lymphoid cells were injected of which 10–20 per cent were labelled immunoblasts. At various times after the injection the rats were exsanguinated and killed and their viscera placed in 10 per cent formol saline. When immunoblasts labelled with ^{3}H-thymidine had been injected, sections were cut from various tissues and autoradiographs were prepared by standard techniques[8]. In this way, it was possible to see the exact microanatomical localization of the injected cells. The quantitative distribution of the injected cells was determined by counting the radioactivity in the organs of rats which had received immunoblasts labelled with ^{125}I-iododeoxyuridine. This thymidine analogue cannot be "re-utilized"[11-13] and has the further advantage that the γ-emission can be counted easily and accurately in a scintillation spectrometer (Packard, model 3002, 'Autogamma'). The results were expressed as a percentage of the total radioactivity injected and also as a specific activity in terms of counts per minute per unit wet weight. Biochemical analyses confirmed that all the activity was associated with macromolecular nucleic acids.

In these experiments twenty recipient rats and one hundred donor rats were used. The distribution of the injected cells was the same whatever the nature of the antigen that had been used to stimulate the donor animals. Immediately after injection many of the injected cells were retained temporarily in the lungs, liver and spleen, but thereafter the number of cells in these organs declined and labelled cells began to appear in the walls of the gut.

Table 1. THE DISTRIBUTION OF RADIOACTIVITY IN VARIOUS ORGANS 24 H AFTER THE INTRAVENOUS INJECTION OF 4×10^7 SYNGENEIC IMMUNOBLASTS LABELLED WITH ^{125}I-IODODEOXYURIDINE

Tissue	Percentage of total activity injected	Specific radio-activity (c.p.m./g)
Lung	0·5	1,500
Liver	2·0	800
Spleen	2·8	16,000
Small gut	21·0	19,500
Large gut	2·7	6,400
Mesenteric lymph nodes	0·5	10,000
Peripheral lymph nodes	0·1	1,000

This process was well established by 1 h and complete at 5 h. After this time the distribution of cells remained relatively constant until 24 h which was the longest time studied (Table 1).

In all rats killed 5 h or more after injection the small gut always contained most of the radioactivity that could be recovered from the viscera, sometimes as much as 40 per cent of the injected dose. This was confirmed by autoradiographic studies which showed a massive infiltration of the lamina propria by labelled cells which were present both at the bottom of the crypts and well up into the villi (Fig. 1). Very few labelled cells gained entry into the Peyer's patches, however, and it was clear that the immunoblasts play no immediate part in populating these tissues. Similarly, the total number of labelled cells in all the other macroscopic lymphoid organs—spleen, lymph nodes and thymus—never exceeded 20 per cent of that in the small gut. In other words, for every immunoblast that reached an actual lymphoid organ four or five went to the small gut. Every organ examined contained some activity and labelled cells were seen in the mucosa of the trachea, the parenchyma of the lung, in the kidney and in the salivary glands.

It was shown originally by Gowans *et al.*[14,15] that when small lymphocytes were injected intravenously most of them entered the spleen, lymph nodes and the Peyer's patches and many of them regained the lymph and continued to recirculate. But the few large lymphoid cells that were injected became localized in the lamina propria of the small gut[16] and this observation has been confirmed[17]. Our results are in accord with these findings and further indicate that most immunoblasts that are generated in lymphoid tissue remote from the gut, in response to varied antigenic stimuli, are destined for the lamina propria of the small gut. Because many of these cells are probably plasmablasts[7] it seems likely that they may be particularly concerned with furnishing the IgA antibodies that protect mucous surfaces in general and the gut in particular[18-22]. In any case the cellular economy of any immune response must involve a substantial investment in the lamina propria of the gut and any assessment of the antibody response will be incomplete unless the antibody content of the mucosa and external secretions is taken into account.

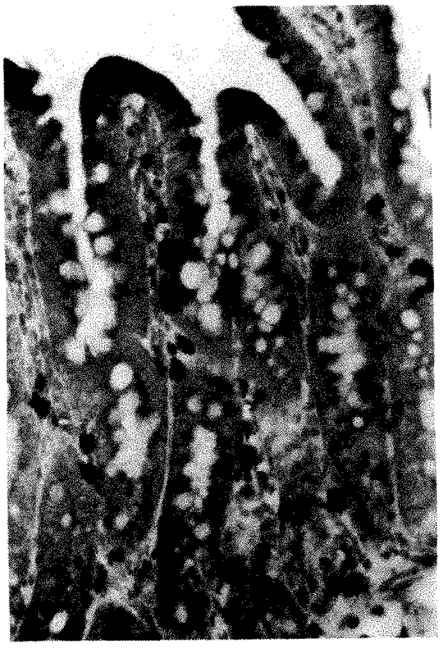

Fig. 1. Photomicrograph of an autoradiograph of a 4 μm section cut from the small gut of a rat 20 h after the intravenous injection of syngeneic immunoblasts that have been labelled *in vitro* with ^{3}H-thymidine. Note the large number of labelled cells in the lamina propria.

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Association of ^{3}H Corticosterone-1,2 with Macromolecules extracted from Brain Cell Nuclei

CORTICOSTERONE, the principal adrenal steroid in the rat, is retained by cell nuclei in the brain of adrenalectomized rats by a process specific to the corticosterone structure[1]. Nuclear binding of hormone is greatest in the hippocampus, where we have previously found the highest tissue concentration of radioactive hormone, but is also significant throughout the rest of the brain[1,2]. The binding of steroid hormone to cell nuclei in other target tissues indicates that the hormone receptor is protein which can be extracted from the nuclei by moderate salt concentrations[3-6]. We now present evidence that hormone within brain cell nuclei is bound to a macromolecular component, probably protein, which can be extracted from isolated nuclei by 0·4 M NaCl.

Male albino rats, adrenalectomized 4–7 days previously[1,2], were injected with 100 μCi of corticosterone (specific activity 40 Ci/mmole, New England Corp., Boston,

Table 1. EXTRACTION OF RADIOACTIVITY AND PROTEIN FROM PURIFIED BRAIN CELL NUCLEI ISOLATED FROM ADRENALECTOMIZED RATS GIVEN 100 μCi ^{3}H-CORTICOSTERONE-1,2 INTRAPERITONEALLY

Structure	Experiment 1 Hippocampus			HTM			Experiment 2 Hippocampus			Cortex		
	c.p.m.	μg protein	s.a.	c.p.m.	μg protein	s.a.	c.p.m.	μg protein	s.a.	c.p.m.	μg protein	s.a.
Buffer wash	—	—	—	19	12	1·583	40	51	0·784	10	68	0·147
0·4 M NaCl	650	264	2·460	48	200	0·240	307	111	2·766	81	231	0·385
0·6 M NaCl	59	216	0·273	11	162	0·068	37	81	0·457	22	140	0·157
1·0 M NaCl	47	88	0·534	6	73	0·081	23	67	0·343	4	72	0·056
2·0 M NaCl	446	1,008	0·442	4	478	0·008	13	411	0·032	5	400	0·012
Residue				27	624	0·043	80	544	0·147	25	712	0·035
Total	1,202	1,576	0·763	115	1,520	0·076	500	1,265	0·395	155	1,623	0·096

The brain regions from which nuclei were isolated are as follows: hippocampus, cerebral cortex, and HTM, a pooled sample consisting of hypothalamus, thalamus, and midbrain. For dissection procedure, see ref. 1. Nuclei from each region were extracted successively with solutions of the salt concentration indicated. s.a.: specific activity, counts per min per microgram of protein.

Mass.) intraperitoneally, or via the carotid artery after acute partial hepatectomy, and were killed by decapitation 1 h later. Nuclei were isolated from whole rat brains and from selected brain regions as described elsewhere[1]. The highly purified nuclei were treated with a standard buffer (0·1 M Tris-HCl, *p*H 7·4; 0·5 mM EDTA, 50 mM $MgCl_2$, 5 mM β-mercaptoethanol) containing various concentrations of sodium chloride solution and centrifuged at 16,000 r.p.m. for 10 min to sediment nuclei and chromatin fragments.

In the first experiments we determined the ionic strength necessary to release the steroid radioactivity from nuclei. Nuclei isolated from brains of adrenalectomized rats injected intraperitoneally with 100 μCi ^{3}H corticosterone were extracted successively with buffer containing 0·4, 0·6, 1·0 or 2·0 M NaCl. Radioactivity was extracted with toluene scintillation fluid and counted in a liquid scintillation counter, and the macromolecular components were precipitated with 10 per cent TCA in 50 per cent ethanol and dissolved in KOH for protein analysis by the method of Lowry *et al.*[7]. Table 1 shows the results of these experiments and it can be seen that the largest amount of radioactivity was extracted by 0·4 M salt. This fraction also had the highest concentration of radioactivity per microgram of protein. As we have reported elsewhere[2], the largest concentration of radioactivity occurred in nuclei from the hippocampus. Lesser but still significant amounts of radioactivity were found in cortex and HTM nuclei, and this showed the same extractability with increasing salt concentration as did the radioactive steroid in hippocampal cell nuclei.

We also used gel filtration on 'Sephadex' to determine the percentage of radioactive hormone, extracted by 0·4 M salt at *p*H 7·4, which is bound to macromolecules. For these experiments, rats were administered 100 μCi corticosterone in saline via the carotid artery after acute removal of the two largest lobes of the liver. These two procedures increased by eight to ten-fold over intraperitoneal injection the amount of ^{3}H-corticosterone which enters the brain. Approximately 1 ml. of extract was placed on a 1·0 × 23 cm column of 'Sephadex *G*-200' suspended in 0·4 M salt in the standard buffer. Thirty ml. of the medium was used to elute the column and sixty fractions were collected with an automatic fraction collector. A typical run is presented in Fig. 1, and shows that nearly 40 per cent of the radioactive hormone is eluted with a protein peak consisting of macromolecules of greater than 800,000 molecular weight[8] which are completely excluded from the 'Sephadex' beads. The remainder of the radioactivity is eluted at the end as free steroid after the rest of the protein has been eluted. In five experiments, an average of 41·6 ± 1·4 per cent of the radioactive hormone was eluted with the macromolecular peak. The presence of free steroid in the extract is not surprising for we have found that, on storage overnight at 4° C, the complex is quite unstable and most of the bound steroid is released from the macromolecular fraction.

It is likely that the peak of macromolecular material in which the bound hormone is eluted (Fig. 1) is an aggregate, possibly artificial, of protein and nucleic acid molecules. Overnight storage of the extract at 4° C has been found to result in a decrease in the highest molecular weight peak and the appearance of lower molecular weight species. Such a dissociation of salt extracts of nuclei into smaller sized fractions by treatment with deoxyribonuclease and ribonuclease has been reported for the oestradiol-binding proteins from the uterus[3]. There is very little DNA in the 0·4 M salt extract of rat brain nuclei, less than 4 μg/mg of protein, and approximately 35 μg of RNA per mg of protein in the extract. Concentrations of DNA and RNA per mg of protein in intact rat brain nuclei are 147 and 53 μg, respectively. Pretreatment of the 0·4 M extracts of rat brain nuclei containing ^{3}H-corticosterone with deoxyribonuclease and ribonuclease has no effect on the binding of hormone, while pretreatment with trypsin for 1 h at 4° C reduces the amount of bound hormone by 40 per cent.

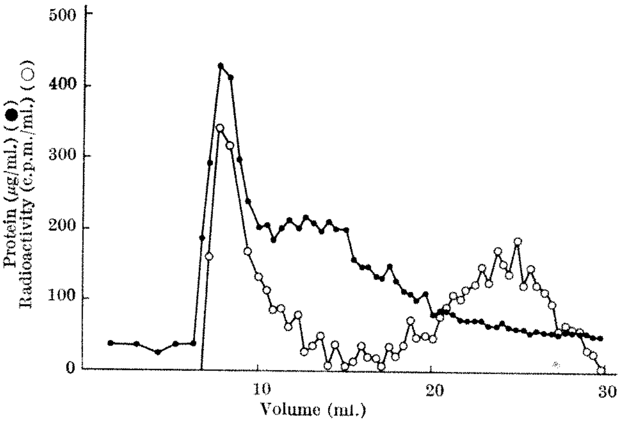

Fig. 1. Elution pattern of protein (●—●) and c.p.m. (○—○) in a 0·4 M NaCl extract of rat brain nuclei labelled with ^{3}H corticosterone-1,2 from a 'Sephadex *G*-200' column. Protein was determined directly on 0·1 ml. of eluate by the method of Lowry *et al.*[7], and radioactivity was extracted directly from 0·1 ml. of eluate with toluene scintillation fluid. The high baseline of protein is due to the colour produced by the standard buffer.

From these results and those from other laboratories on nuclear hormone binding factors, it seems highly probable that the binding substance is a protein of a type which is believed to have a regulatory function in the cell nucleus over RNA and subsequent protein synthesis[9,10]. We are at present attempting to purify the corticosterone-binding macromolecules from brain cell nuclei and to determine a regulatory role for this substance.

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Shared Viral Antigen of Mammalian Leukaemia Viruses

IN 1966, Geering, Old and Boyse[1] identified group-specific (gs) antigens of murine leukaemia viruses (MuLV) in immunoprecipitation tests with antigen in the form of disrupted virions or extracts of infected tissue. Antisera containing a wide range of precipitating MuLV antibodies are obtained from inbred rats bearing large subcutaneous transplants of syngeneic leukaemias induced by wild-type Gross virus[1]. Usually a large number of rats have to be screened in order to find one with antibody to the complete set of MuLV antigens known to be demonstrable by immunoprecipitation. Rats which have both a massive local tumour and metastasis to local lymph nodes, and which have survived for a comparatively long time after they received the leukaemic transplants, are most likely to yield such antisera.

Gregoriades and Old isolated and partially characterized one of the soluble gs antigens, MuLV-gs1 (ref. 2); a gs antigen with similar properties has been studied by Schäfer *et al.*[3]. Another of the MuLV gs antigens, MuLV-gs3, is now known to be shared by the feline leukaemia virus (FeLV)[4]. Leukaemia viruses of C or enveloped A type[5] have been found in primary and transplanted tumours of the rat and hamster (refs. 6–12 and personal communications from P. Sarma and H. Chopra) as well as in cats and mice; Fig. 1 illustrates their morphological similarity to MuLV and FeLV. We now report that antigen gs3 is present in these viruses also.

The rat virus was obtained from the peritoneal fluid of rats bearing the Novikoff ascites hepatoma, and the hamster virus from washings of minced D–9 lymphosarcoma[11,12]. In each case the virus was isolated by density gradient centrifugation in potassium tartrate as previously described for MuLV and FeLV[2,4,13]. (The band is at 1·3 g/cm^3 for D–9 virus[14] and about 1·15 g/cm^3 for Novikoff virus.) Soluble components were released by treatment with ether[1]. Ouchterlony tests were performed in 2 per cent agar ('Immunoplate' pattern C, Hyland, Los Angeles).

Fig. 2 illustrates the presence of gs3 in the mouse, cat, hamster and rat leukaemia viruses. The major antigens of MuLV, gs1 and gs2 (fused heavy band in Fig. 2), are absent from the cat, hamster and rat viruses; gs3 antigen in the MuLV preparation is obscured in this illustration by the intense reaction of the other gs components. No serological cross-reaction between the mammalian and avian leukaemia viruses has been observed (Fig. 2 and refs. 4 and 14).

When this shared antigen was first identified in MuLV it was designated MuLV-gs3. Because the same antigen has now been found in two other species of Rodentia as well as in one species of Carnivore, the notation is no longer appropriate, for the group of viruses which it characterizes is not confined to the mouse. But perhaps the problem of re-naming it should be dealt with later when more is known about the comparative immunology of mammalian leukaemia viruses.

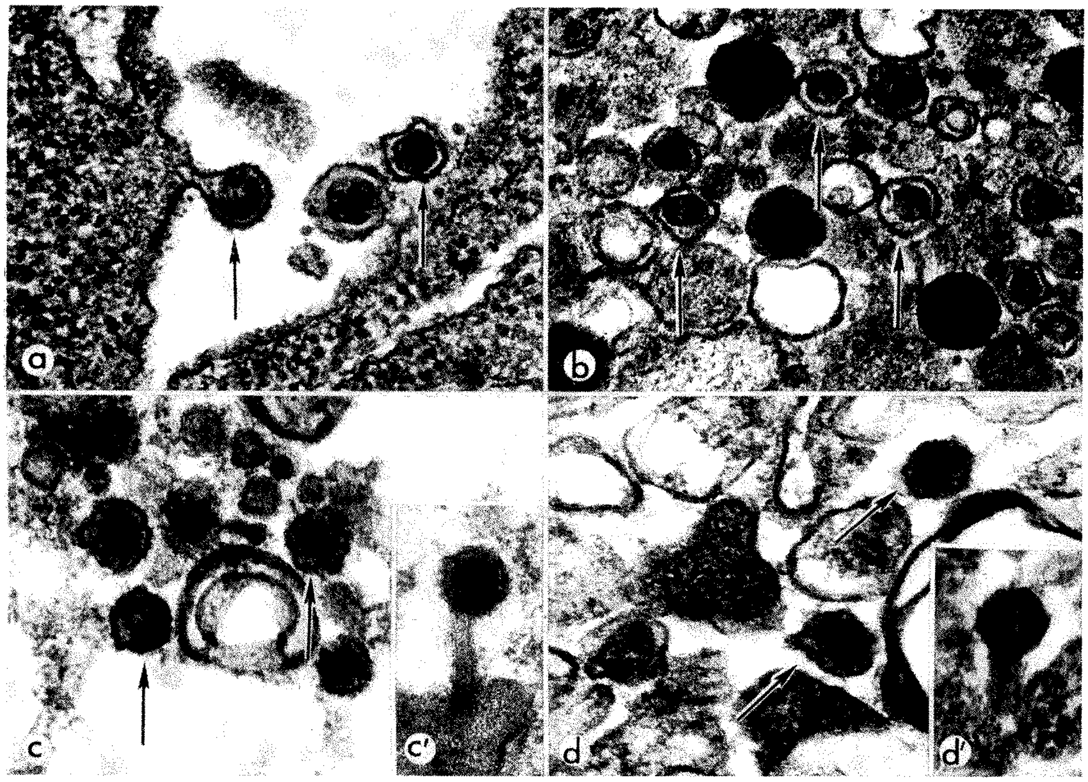

Fig. 1. Virus of type C or type A morphology occurring in different mammalian species (× 69,600). (*a*) C57Bl/6 mouse leukaemia induced by passage A Gross virus; C-type particles and an enveloped A-type particle budding from the cell surface. (*b*) Novikoff rat hepatoma, ascitic form; many C-type particles in a pellet prepared from ascitic fluid. (*c*) Cat lymphosarcoma; C-type and enveloped A-type particles in the intercellular space; insert (*c'*) shows budding figure of an enveloped A-type particle. (*d*) Hamster D–9 lymphosarcoma; enveloped A-type particles; insert (*d'*) shows budding figure of an enveloped A-type particle. Samples were fixed in 1 per cent glutaraldehyde and then in 1 per cent OsO_4, and embedded in 'Epon'. Thin sections were stained with uranyl acetate and lead citrate, and examined with a Siemens 'Elmiskop 1A' electron microscope.

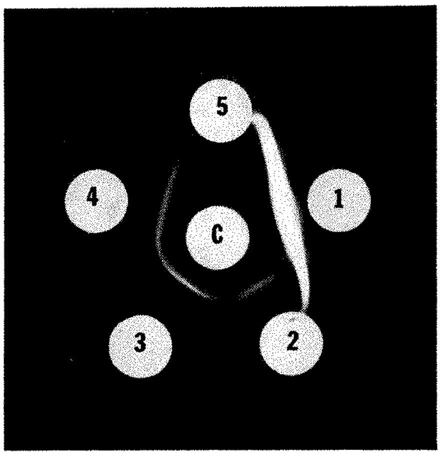

Fig. 2. Centre: MuLV antiserum. Peripheral wells: ether-disrupted virus from (1) mouse (MuLV); (2) cat (FeLV); (3) hamster (D-9 lymphosarcoma); (4) rat (Novikoff hepatoma); and (5) chicken (BAI strain A of avian myeloblastosis virus). The antigen shared by the mammalian leukaemia viruses is gs3.

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Suppression of Autoimmune Disease in NZB and $(NZB\times NZW)F_1$ Hybrid Mice by Infection with Malaria

VARIOUS immunological changes have been observed in many apparently healthy subjects living in tropical regions that are probably related to multiple parasitic infections from childhood[1]. Consideration of this in conjunction with the rarity of autoimmune disease in tropical Africa, for example Western Nigeria[2], led to the suggestion that the immunological effects of repeated parasitic infection might have some protective action against the development of autoimmune disease[2]. To investigate this hypothesis we have studied the effects of infection with malaria on the spontaneous autoimmune disease of NZB mice and the $(NZB\times NZW)F_1$ hybrid mice (B/W mice). Twenty-one NZB mice and thirteen female B/W mice from the Taplow colony were infected, when one month old, with the rodent malaria parasite *Plasmodium berghei yoelii*[3] by the intraperitoneal injection of 1×10^6 parasites derived from a heavily infected mouse of the same strain. The malaria strain was not contaminated with *Eperythrozoon coccoides*. Parasitaemia was monitored by the examination of Giemsa stained blood films made from the tails of the mice every second day during the acute phase of the infection and at longer intervals thereafter. To determine whether low parasitaemia was persisting, heparinized blood samples were collected from each mouse 4 months after infection and pooled samples injected into young BALB/c mice.

Blood and 24 h urine samples were collected from all infected mice at monthly intervals. Direct Coombs tests were performed using a rabbit anti-mouse globulin antiserum which gave strong lines with mouse IgG and IgM on immunoelectrophoresis. Sera were tested for antinuclear factor at a dilution of 1 : 10 using rat liver as substrate. A quantitative protein estimation was carried out on an aliquot of each 24 h urine sample using sulphosalicylic acid. Cryostat sections from snap-frozen kidneys removed from some of the mice were examined for the presence of immunoglobulin deposits by direct immunofluorescence.

Table 1. FEATURES OF THE PARASITAEMIA OF NZB AND B/W MICE INFECTED WITH *Plasmodium berghei yoelii*

Mouse strain	Peak parasitaemia (Parasites/10^4 erythrocytes) Mean	Range	Duration of parasitaemia (days) Mean	Range
NZB	$1,570\pm 273$	600–2,920	$19·2\pm 0·44$	18–22
B/W	$1,920\pm 418$	718–5,350	$12·6\pm 0·22$	12–14

The pattern of disease occurring in the infected mice was compared with that previously observed in control animals from the Taplow colony. Four control mice from each strain were studied in parallel with the mice infected with malaria. The pattern of disease in these animals closely followed that previously observed in the larger control groups studied at Taplow. Five malaria-infected NZB mice and two infected B/W mice were killed within the first three months of life in order that their kidneys could be examined for features of complex nephritis.

The parasitological features of the malaria infection in the two strains of mice are shown in Table 1. All the mice of both strains inoculated with *P. berghei yoelii* became infected. The NZB mice had a longer period of parasitaemia than the B/W mice but the latter strain had a higher peak parasitaemia. No parasitaemia resulted from the sub-inoculation of pooled blood obtained from any of the infected mice 4 months after infection, indicating that persisting infection was not present.

Control female B/W mice developed severe proteinuria when 6–8 months old and 97 per cent had died by the age of 11 months (Fig. 1). At the age of 9 months 58 per cent of the control mice gave a positive test for antinuclear factor. By contrast the eleven B/W mice infected with malaria were all alive at 11 months and none had developed proteinuria or a positive test for homogeneous antinuclear factor. The differences between control and infected mice with regard to mortality at 11 months, proteinuria at 11 months and incidence of positive antinuclear factor at 9 months are all statistically significant ($P<0·01$).

Three of the malaria infected B/W mice were killed at 12 months. The kidneys of these mice contained only small deposists of immunoglobulin by contrast with the heavy deposits seen in the kidneys of control animals examined at 7 or 8 months.

The onset of a positive Coombs test in the NZB mice infected with malaria was delayed in comparison with the controls (Fig. 2). The number of malaria infected mice with a positive Coombs test at 9 months (four of the sixteen) is significantly lower than the number of control animals with a positive test at the same age (sixteen out of seventeen) ($P < 0.01$). By 12 months, however, no significant difference was evident in the proportion of mice with a positive Coombs test between the malaria infected mice and the controls. At 12 months the mean packed cell volume was similar in both groups.

These experiments indicate that the early infection with the rodent malaria parasite *P. berghei yoelii* is associated with a delay in the onset of haemolytic anaemia in NZB mice and is associated with protection against the development of renal disease in B/W mice. The mechanism by which the malaria infection modifies the course of these autoimmune diseases remains a matter for speculation. Although the malaria strain used in the present study had been passaged in blood only twice since mosquito passage, it may have been contaminated by passenger viruses. It is therefore possible that the protective effect of the malaria infection was a result of these viral contaminants rather than of the malaria parasite itself. There is evidence that viral infection is important in the induction of autoimmune disease in NZB mice[4], and interferon production by the malaria parasite or its contaminants may have had a deleterious effect on the causative virus. On the other hand, the failure of the interferon inducer 'Statolon' to influence the onset of Coombs positivity in NZB mice is evidence against this hypothesis[5].

At the height of malaria parasitaemia the immune response of infected mice to sheep cells is reduced[6], but it seems unlikely that suppression of the effector immune response would persist for a prolonged period after recovery from the parasitaemia. It seems more likely that infection with malaria enables NZB and B/W mice to maintain tolerance to an antigen, possibly of viral origin, which usually induces a harmful immune response.

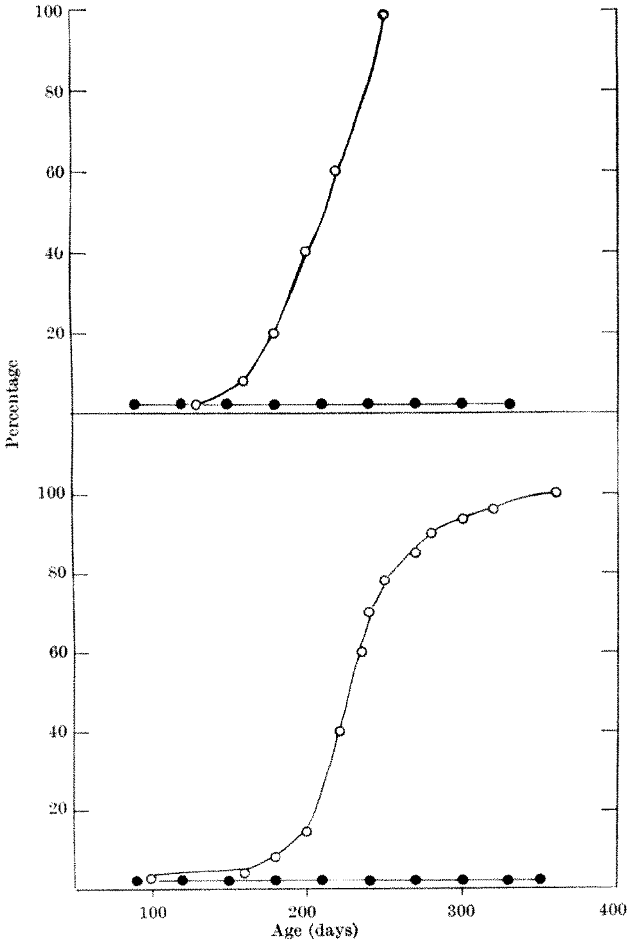

Fig. 1. Top, incidence of proteinuria greater than 10 mg/24 h in eleven malaria infected female B/W mice and in forty female controls. Bottom, cumulative mortality curve for eleven malaria infected female B/W mice and 101 female controls. ●—●, Mice infected with *P. berghei yoelii*; ○—○, controls.

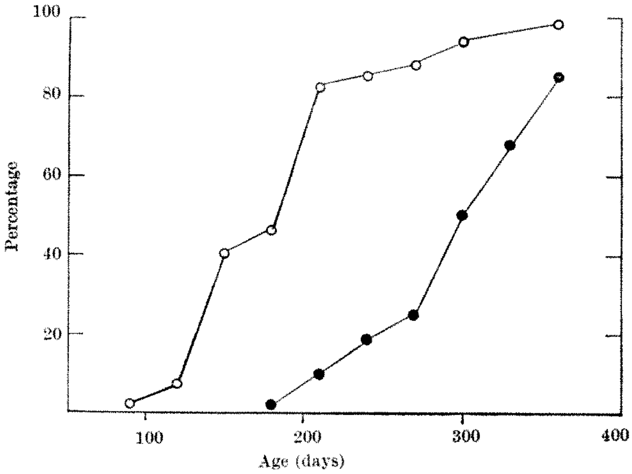

Fig. 2. Incidence of a positive Coombs test in sixteen malaria infected NZB mice and in seventeen controls. ●—●, Mice infected with *P. berghei yoelii*; ○—○, controls.

Considerable caution is needed in extrapolating the results of these animal experiments to a consideration of human disease. They do, however, support the idea that repeated parasitic infections may have some protective action against the development of autoimmune disease in man. Elucidation of the mechanism by which infection with *P. berghei yoelii* suppresses autoimmune disease in mice might indicate a new approach to the treatment of human autoimmune disease.

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Changes in Leptomonads of *Leishmania tropica* grown in Media containing Immune Serum

THE method for differentiating strains of *Leishmania*, introduced by Adler[1,2], is to grow them on Locke–serum–agar (semi-solid medium) or on Rugai's medium to which homologous or heterologous immune serum is added. After 3 or 4 days of cultivation, the formation of large

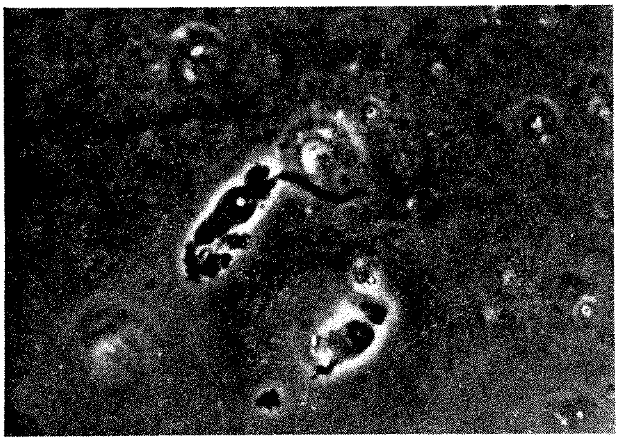

Fig. 1. *Leishmania tropica* on homologous immune serum 1 : 20. Fresh preparation; 6 h culture; ×1,000, phase-contrast. Note pyriform cell, enlarged flagellar vacuole, vesicle at base of flagellum.

irregular, aflagellar cell masses, containing an accumulation of various granules, can be observed. These cell masses were initially considered to be syncytia from which flagellar forms were assumed to re-differentiate at a later stage. Observations made on younger cultures aroused some doubts, however, regarding the nature of these syncytia (personal communication of S. Adler) and we therefore decided to follow up changes produced in the leptomonads by examination of cultures at short intervals, especially during earlier stages of cultivation.

For experimental purposes, flagellates form 4 to 5 day old cultures of the Ein Gedi II strain, routinely maintained at 28° C on Locke–serum–agar with rabbit haemoglobin, were inoculated on Locke–serum–agar or on Rugai's medium to which immune rabbit serum was added in the ratio 1 : 20 (normal serum in control media). The immune serum was prepared in rabbits by six intravenous injections of flagellates at 6 day intervals. The rabbits were bled 10 days after the last injection. Observations were carried out by simple and phase microscopy directly on drop samples of fresh, untreated, or trypan-blue stained preparations, as well as on air-dried smears stained with May–Grünwald stain.

The observed changes involved extracellular and intracellular phenomena. In the medium, a precipitate consisting of flake-like aggregates formed immediately on addition of unwashed leptomonads. In some sera the precipitate persisted for several days, the aggregates enveloping the cells; in other sera the minute droplets which constituted the aggregates coalesced to form hyaline bodies, encompassing single cells or cell groups. The precipitate is a complex of exo-antigens[3] or metabolites accumulating in the maintenance medium, and the antibody in the experimental medium. This could be proved in the first 30 h of cultivation by staining the washed precipitate with a sheep anti-rabbit globulin, conjugated with fluorescein isothiocyanate. By the third day the complex apparently underwent some changes, for it no longer stained with the conjugated globulin. Fluorescence was also observed on the cell surface. No precipitate was formed when the inoculum was washed several times with phosphate-buffered saline before it was added to the experimental medium, whereby changes in the cells were delayed for several hours.

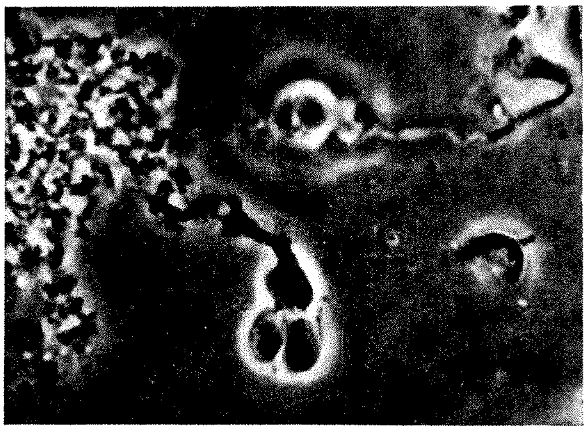

Fig. 2. *Leishmania tropica* on homologous immune serum 1 : 20. Fresh preparation; 16 h culture; ×4,200, phase-contrast, electronic flash. Cell after division. Note daughter cells held together by vesicle formed at base of flagellum and extending almost to tip; precipitate at left.

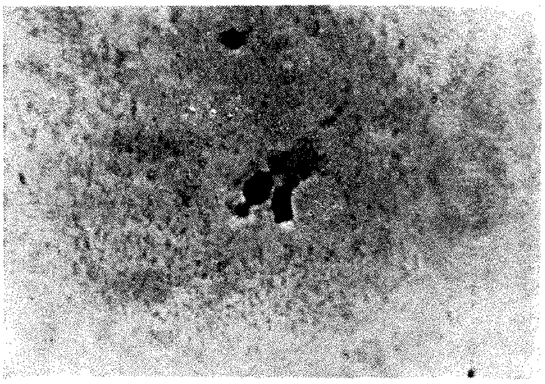

Fig. 3. *Leishmania tropica* on homologous immune serum 1 : 20. May–Grünwald phase-contrast; ×1,000. Same stage as Fig. 2. Note vesicle moulded around original flagellum; flagella of daughter cells embedded in vesicle.

Intracellular changes involved an almost immediate immobilization of the cell body and gradual slowing of flagellar movement until it ceased. The cells became pyriform, the flagellar vacuola enlarged and a small spherical vesicle was formed around the base of the flagellum (Fig. 1). The vesicle then spread and finally encircled the entire flagellum. After the first division, the daughter cells, observed by phase microscopy, appeared spherical and held together by the vesicle and the flagellum (Fig. 2). On staining with May–Grünwald stain, however, it became evident that each spherical daughter cell had a separate flagellum (Fig. 3). As cell division continued, groups of cells, the progeny of a single leptomonad, were formed, each with its own flagellum, all held together by the vesicle. Such groups of cells, embedded in the flake-like precipitate or united by hyaline material, formed the cell clumps, originally described as "syncytial" masses (Fig. 4). The granular centre of the clump consisted of precipitate, detached flagella and cell debris. On the surface of the clumped cell, division continued and after 3 to 4 days normal, elongated cells were seen. Adler considered the emergence of these cells as resulting from a decrease of antibody concentration in the medium[2].

The presence of immune serum in the medium does not interfere with normal mitosis. Descendants of a single cell are held together by the vesicle. The immune serum, however, presumably produces alterations in the cell membrane which cause leakage with vesicle formation and rounding of the cell body; it may, moreover, contribute to changes in the intracellular metabolic processes[4] which would lead to an accumulation of various granules as observed by Adler[2].

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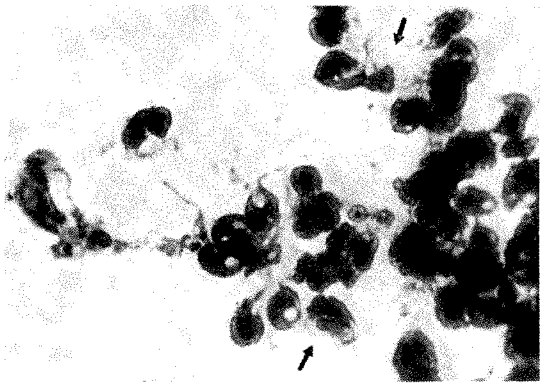

Fig. 4. *Leishmania tropica* on homologous immune serum 1 : 20. May-Grünwald, phase-contrast; ×1,000; 42 h culture. Clusters of cells embedded in faintly stained precipitate. Note (upper arrow) two groups of cells each united by vesicle; hyaline precipitate (lower arrow); enlarged flagellar vacuoles.

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Changes in the Electrical Impedance of Blood during Coagulation

Reports on the impedance changes of blood during coagulation have been conflicting, and many have concluded that there is no significant impedance change which could be related to the coagulation process[1-5]. In 1948, however, Rosenthal and Tobias[6], using improved techniques, demonstrated a significant increase in impedance following coagulation, but the technique never came into practical use. Moreover, though some workers confirmed the observations[7], others claimed to obtain conflicting results[8].

Earlier difficulties in measuring blood impedance changes during coagulation were due not only to insensitive instruments[9] but also to the masking effects of cell sedimentation, particle disintegration, temperature changes, agitation, and so on[2,5,10-12]. Practical application was therefore abandoned[9], and the only device using electrical conductivity in any way is the "fibrin-switch" which is, essentially, a mechanical device[13,14].

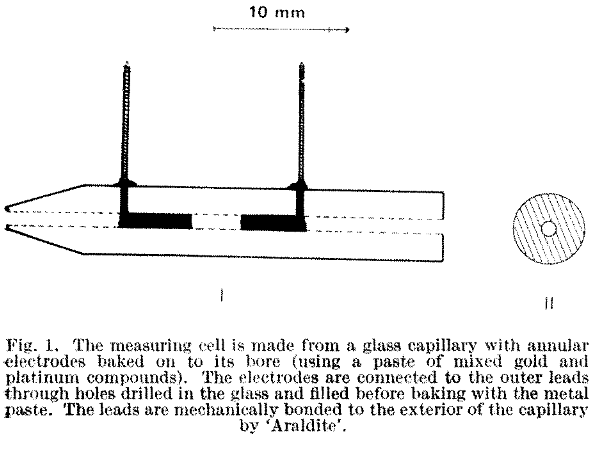

Fig. 1. The measuring cell is made from a glass capillary with annular electrodes baked on to its bore (using a paste of mixed gold and platinum compounds). The electrodes are connected to the outer leads through holes drilled in the glass and filled before baking with the metal paste. The leads are mechanically bonded to the exterior of the capillary by 'Araldite'.

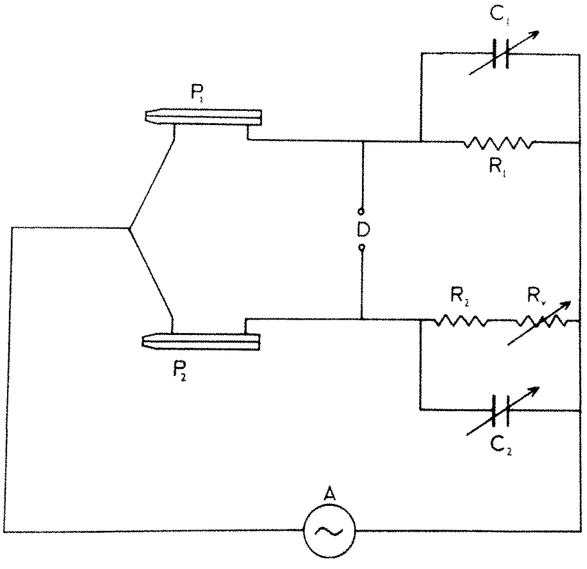

Fig. 2. The measuring bridge. P₁ Reference (heparinized) cell; P₂, measuring cell; R₁, fixed resistor of 15 kΩ; R₂, fixed resistor of 10 kΩ; Rv, 10 turn helipot of 10 kΩ; C₁ and C₂, variable capacitors of 250 pF; D, oscilloscope having sensitivity of 1 mV/cm; A, oscillator providing 1·2 V, 10 kHz sinusoidal tension.

A new principle is described here, which enables impedance changes caused by the clotting mechansim to be isolated from those caused by other processes in the blood. It allows continuous undisturbed impedance monitoring of the blood sample with a clear indication of the clotting time. The principle on which this work is based may, moreover, be useful in studies on cultures of microorganisms and the effect of antibiotics on such cultures; studies of antigen–antibody reactions; and other investigations of reactions in liquid systems.

The electrical impedance of a cell (Fig. 1) containing the clotting blood is measured in a bridge circuit (Fig. 2) in which the balancing resistance consists of a similar cell, containing blood from the same sample, but in which coagulation has been prevented by the addition of about 1 µl. of 5,000 U/ml. heparin. All impedance changes are therefore balanced but for those resulting from coagulation. The method also excludes impedance variations arising from individual differences in haematocrit, electrolyte concentration, urea concentration, and so on, all of which affect impedance of the blood[12].

The bridge was supplied with 1·2 V, 10 kHz sinusoidal tension. The blood, collected from finger puncture in a pipette, was divided between the measuring cells, the reference cell (containing the heparin) being filled last. In early experiments, the bridge was continually balanced by adjusting the helipot R_2 and the variable capacitors at predetermined time intervals (for example, 30 s) and the results were obtained from the helipot scale. Later, the output voltage from the bridge was recorded on a continuously running chart recorder with the bridge offset by reducing R_2 from the original balance point so that the balance point was avoided during recording.

The variation of electrical impedance of a blood sample during coagulation was highly reproducible in several hundred tests. Fig. 3 illustrates the typical decrease in the impedance during the first 12 min, followed by a less steep increase. Both the descending and ascending parts of the curve contained secondary steps of characteristic form.

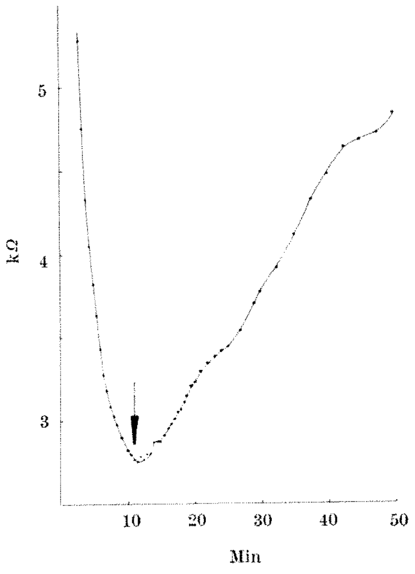

Fig. 3. The changes in blood impedance during coagulation. The ordinate represents the values of the helipot as the bridge was continually balanced. The arrow points to the coagulation time of the control samples.

The relationship of the curve to the coagulation process was examined as follows: A blood sample, collected in a pipette, was divided among five cells; first to be filled was the bridge cell in which the clotting was to occur, then three identical cells which served as controls and finally the reference bridge cell containing heparin. All cells were filled within 30 s. While the impedance changes were recorded, the control cells were checked for the appearance of clot, by probing the blood in the first cell with a wire hook at 30 s intervals. After clotting of the blood in the first control cell, the other two were successively examined. The time at which a solid clot was found in the third cell is represented in Fig. 3 (arrow). In a series of such experiments clotting was within 30 s of the minimum of the impedance curve. Improvement of the technique—by consistent techniques of blood collection and manipulation of the cells—reduced the scatter of the minima. Experiments on different days on blood of the same subject did, however, show variations, which are probably physiological.

Impedance measurements were performed also in experiments where: (*a*) both sample and reference cells were filled with clotting blood; (*b*) both cells were filled with heparinized blood; (*c*) the reference cell was replaced by a constant resistor while the sample cell was filled with clotting blood. The curves obtained in experiments (*a*) and (*b*) were relatively flat and the fluctuations recorded a higher amplification were not reproducible. The curves obtained in experiment (*c*) had various forms which were not reproducible and had no specific pattern which could be related to the coagulation process.

This method enables interpretation of some of the conflicting results obtained by previous workers. Rosenthal and Tobias were able to obtain curves which closely resemble the ascending branch of the curve displayed in the present work. It seems, moreover, that their data show the prominent step of the ascending branch, but in plotting the curves they preferred to ignore this step (Fig. 5 of ref. 6). Their readings also correspond to part of the descending branch, but they assumed these higher readings to be caused by temperature variations or by insufficient time for thorough wetting of the electrodes, and excluded them (Figs. 4 and 5, ref. 6). They concluded therefore that before clot formation there is no significant change in resistance. On the other hand, Mungall *et al.*[8] obtained initial decrease in impedance, and probably being unaware of the data ignored by Rosenthal and Tobias, interpreted their findings as conflicting with those of the latter. Errors in earlier studies can also be attributed to the neglect of factors such as agitation; the ratio of the volume of the sample to its area contacting the container; surface properties; and temperature. Unless these factors are taken into account[15-18], the value of any correlation is doubtful.

The causes for the typical impedance changes displayed in Fig. 3 are not certain. The curve might represent several processes which affect the impedance, some of them in opposing directions. Thus, the beginning of the clotting process might be dominated by disintegration of corpuscles (platelets) and degradation of certain proteins which would increase the number and the mobility of the charged particles. The increasing impedance following coagulation has been related to clot retraction and solidification[6,7,19]. The formation of a fibrin network, the trapping of blood corpuscles in this network and clot retraction might all decrease the motility of the charged particles, while variations in the effect of such processes may lead to the secondary steps and the fluctuations in the curves.

The method described may allow a convenient and accurate test for coagulation studies, particularly in connexion with the control of anticoagulant therapy where the lack of such a test may have been the obstacle to progress[20].

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Phospholipid—Retinaldehyde Complex absorbing at 500 nm

COMPLEXES between retinaldehyde and ethanolamine phosphoglyceride (EPG)[1] have been reported by several workers[2-5], and Daemen and Bonting[4] have shown one of these (λ_{max} 450 nm in anhydrous chloroform) to be an internally protonated Schiff's base. We call this complex I. Adams[2] described an additional product, formed by combining retinaldehyde with EPG, the λ_{max} of which is 500 nm (Fig. 1). This substance, which we call complex II, is characterized here. EPG is one of the two principal phospholipids in bovine rhodopsin[6], so such complexes may well exist in the visual system and, indeed, have been identified in retinal extracts[3,5,7]. Our experimental observations indicate a direct relationship between the complex absorbing near 450 nm (I) and that absorbing at 500 nm (II). The molecular features required to form complex II are enumerated.

Addition of EPG in anhydrous chloroform–methanol to retinaldehyde in the same solvent at room temperature produces an immediate colour change characterized by a rapid increase in absorption in the 450–470 nm region. An additional increase begins at 500 nm within 30 min. Absorption in both regions continues to increase, reaching equilibrium after 3 h. We have observed this reaction using phospholipids extracted from plants, eggs or cattle retina, and combined with the following retinaldehyde isomers: all-*trans*, 9-*cis* and 13-*cis*. When these complexes are subjected to thin layer chromatography (TLC) (Fig. 1), I and II separate. Complex I has an R_F value of about 0·10 and is red–orange with absorption maxima at 458 and 340 nm. Complex II is rose in colour, has an R_F value of 0·23 and absorption maxima at 500 and 340 nm. Both substances can be eluted with chloroform–methanol (2 : 1). We confirm Daemen's finding (personal communication) that complex I is light-sensitive; when allowed to stand in room light, the red–orange colour reverts to the yellow of retinaldehyde within 1 h. In the dark the colour is stable. Complex II is much less sensitive to light, requiring several days to lose its colour. The reactants form I and II by absorbing either heat or light: mixtures of retinaldehyde and phospholipid at − 60° C show no evidence of either I or II until the mixture is exposed to light. While complex I can form at − 60° C in light, no complex II is formed unless the temperature is raised to 0° C or above. A solution allowed to react at low temperature in the light for 20 h and then warmed to room temperature produces an intense red–orange colour in a shorter space of time than a solution allowed to react at room temperature. No differences were noted between reactions in a normal atmosphere and those under nitrogen using degassed solvents.

When anhydrous methanolic HCl was added to either I or II, after elution from TLC, slight spectroscopic changes were seen only in II. The 500 nm peak shifted 3–5 nm toward the blue and the 340 nm peak about 5 nm toward the red. Addition of anhydrous methanolic HCl to I produced no changes. The addition of methanolic KOH (anhydrous) to either I or II brought about a rapid spectral change with both maxima shifting to 387 nm (presumably retinaldehyde). The initial changes in the spectrum of II, however, indicated the presence of I during the transition. In view of this, a sample of I eluted from TLC was concentrated by evaporation and on rechromatography showed the presence of II.

The only variable component of a mixture of retinaldehyde and EPG is the fatty acid content. Although equimolar samples were prepared, it seems that the percentage of fatty acid esters recovered from I is greater than from II as shown by gas liquid chromatography (GLC) (Table 1). The discrepancy suggests that II must be incompletely methylated. The methylating procedure of Morrison and Smith[8] (BF_3–methanol treatment at 100° C for 30 min) produced a brownish solution. On addition of water to hydrolyse the BF_3, the benzene layer became rose coloured, indicating that methylation was not complete. Attempts to methylate more completely were made using sodium methoxide (0·5 per cent in methanol)[9]. No improvement was noted over the results obtained with BF_3–methanol. Hydrolysis of II with methanolic KOH led to the disappearance of the rose colour; after exact neutralization with HCl, however, a rose coloured precipitate formed, presumably the potassium salt. The remaining solution contained no EPG.

Less unsaturated fatty acids were recovered from II than I (Table 1), suggesting that these are less available for methylation. The role of unsaturated fatty acids in the formation of II was examined using compounds similar to EPG from natural sources. Dipalmitoyl-L-α-cephalin (Mann Research Laboratories, New York), a completely saturated fatty acid phospholipid, produced a good yield of a II type complex; analysis of this cephalin by GLC showed, however, that it contained at least 10 per cent $C_{16:1}$ fatty acid. Distearoyl EPG was synthesized[10] and reacted with retinaldehyde: it produced a trace amount of II but GLC showed this also contained trace contaminants of 16 : 1 as well as 18 : 1 acids. Hydrogenation of the L-α-cephalin by the platinum–borohydride technique (Brown Hydroanalyser, Delmar Scientific Laboratories, Maywood, Illinois) yielded a substance which contained no unsaturated fatty acids detectable by GLC. When reacted with retinaldehyde it produced good yields of I but no II detectable by TLC. This confirms the necessity for an unsaturated fatty acid component of EPG to form II.

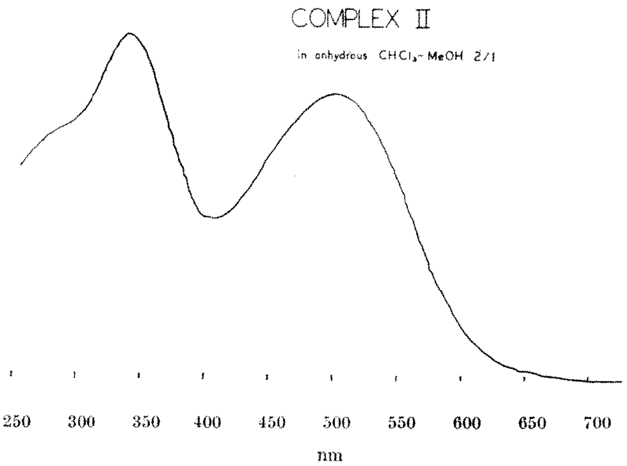

Fig. 1. Complex II was isolated on silica gel impregnated glass fibre sheets ('ITLC-SAF', Gelman Instrument Co., Ann Arbor, Michigan) by a two-dimensional system: 9 per cent methanol in chloroform followed by 2 per cent acetonitrile in chloroform. Pieces were excised and extracted with chloroform : methanol (2 : 1) for spectroscopy on a Cary 14 instrument.

The role of other molecular features of retinaldehyde which may be involved in the formation of II was examined using the vinylogous series, $C_6H_5(CH{=}CH)_nCHO$ (ref. 11). Compared with retinaldehyde, this series has (*a*) no methyl groups and (*b*) other conjugated chain lengths. For $n = 1$ to 6 we found that each compound of the series formed complex I but only those compounds with $n > 4$ formed II. The presence of methyl groups is not needed to form either I or II, even though a minimal chain length is required to form II. This leads us to characterize II as arising from I by an intramolecular interaction. The requirement for an unsaturated fatty acid residue in EPG suggests a π–π association between the isolated double bond of the unsaturated fatty acid and the retinaldehyde residue. Interactions of π orbitals (among them charge transfer processes) have been invoked by several authors[12–15] for biological systems, including rhodopsin; compounds like II are consistent with Dartnall's concept

Table 1. FATTY ACID METHYL ESTERS RECOVERED FROM HYDROLYSED EPG-RETINALDEHYDE COMPLEXES I AND II BY GLC

Preparation	Total fatty acid methyl esters	Oleic (18 : 1) / Stearic (18 : 0)		Palmitoleic (16 : 1) / Palmitic (16 : 0)	
	II/I	I	II	I	II
1	0·80	1·70	0·82	3·32	0·67
2	0·68	2·70	1·60	0·33	0·19
3	0·75	2·10	1·00	No C_{16} acids*	
4	0·86	2·40	1·30.	5·50	2·00

* Preparation using synthetic di-oleyl EPG.

The principal fatty acid methyl esters (about 80 per cent) occur in both I and II as C_{18} and C_{16} fractions. The last four columns show that in both fractions the recovery of unsaturated fatty acid methyl esters is relatively greater from I than from II.

of multiple orbital interactions[16]. Calculations of molecular orbital parameters and considerations of stereochemical relationships further confirm the spectral data and structural conclusions discussed here.

The distinctive molecular features involved in the formation of complex II are (1) the primary amino group of the EPG which, as in the case of complex I, participates in the formation of a Schiff's base with the aldehyde group of retinaldehyde; (2) the unsaturated fatty acid residue of EPG which may associate with the retinaldehyde through a π–π interaction; (3) the length of the conjugated chain of the retinaldehyde, which is at the lower limit ($n=4$) in the limited series of ω-phenyl-enals that were found to form II type substances when reacted with EPG. The lack of a specific stereochemical configuration is not important because II can be formed by at least three of the known retinaldehyde isomers.

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Conformational Changes of Mitochondria associated with Uncoupling of Oxidative Phosphorylation *in vivo* and *in vitro*

BIOCHEMICAL and electron microscopical studies have revealed[1-5] that isolated mitochondria fixed in different respiratory states display characteristic changes in their ultrastructure, and it is believed that conformational modifications of the inner membrane are fundamental to energy transfer in mitochondria[1,5]. We now report the results of a study on uncoupled mitochondria in cultured chick embryo heart myoblasts fixed *in situ*, and on isolated rat liver mitochondria uncoupled *in vitro* and examined unfixed with the electron microscope.

One day old hanging drop primary cultures of myoblasts were used. Suspended in Tyrode solution the cultures showed an oxygen consumption of about 9·5 µl./mg dry weight/h and their respiration was 50–70 per cent coupled to phosphorylation, as demonstrated by oligomycin inhibition and reversal of inhibition by 2,4-dinitrophenol. The addition of uncoupling agents to the suspension caused an increase from 50 to 100 per cent of the cell respiration.

The cultures were placed in small flat chambers containing one of the following uncoupling agents dissolved in Tyrode solution; 100 µM dinitrophenol, 100 µM 4,6-dinitro-*o*-cresol, 100 µM pentachlorophenol, 100 µM dicoumarol, or 1 µM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) and incubated at 38° C for 60 min. They were then fixed in 2·5 per cent glutaraldehyde in Tyrode solution for 30 min at 0° C, post-fixed in 1 per cent osmium tetroxide in Tyrode solution for 60 min at 0° C and prepared for electron microscopy as already described[6]. All procedures were strictly standardized and the mitochondria of cells under definite experimental conditions consistently had the same ultrastructural features. (For a description of mitochondria in chick embryo heart cultured myoblasts see ref. 7.)

Two orders of changes were observed in the mitochondria of the cells treated with uncouplers of oxidative phosphorylation: (1) modifications which can be considered as physiological modulations because they are seen in the cell in normal conditions; and (2) pathological changes, which consist of polymorphous modifications of shape and ultrastructure of the mitochondria that are not usually seen in normal cells. Only the former type of modifications will be described.

In all the cells, the five uncoupling agents caused the following changes in 40–70 per cent of the mitochondria: the matrix showed an increased opacity to electrons and its structure appeared more homogeneous; the limits between the inner membrane and the matrix were much less evident or even disappeared; the intracristal spaces were increased. This ultrastructural transformation of the mitochondrial inner sac seemed to be somewhat similar to that described by Hackenbrock[1] as "condensed", in isolated liver mitochondria in respiratory state 3 (as defined by Chance and Williams[8]). Further, the mitochondria were thinner and their mean diameter was from

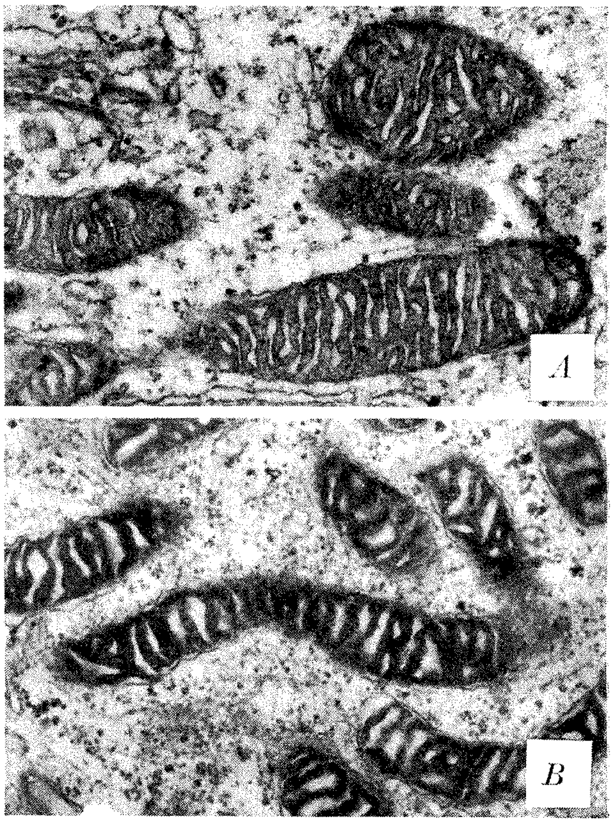

Fig. 1. *A*, Normal mitochondria in chick embryo heart myoblasts cultured *in vitro* and treated with Tyrode solution for 1 h at 38° C. × 30,000. *B*, Mitochondria in chick embryo heart myoblasts cultured *in vitro* and treated with 100 µM dicoumarol in Tyrode solution for 1 h at 38° C. Shrinking of the inner mitochondrial sac and decrease of the mitochondrial diameter. × 30,000.

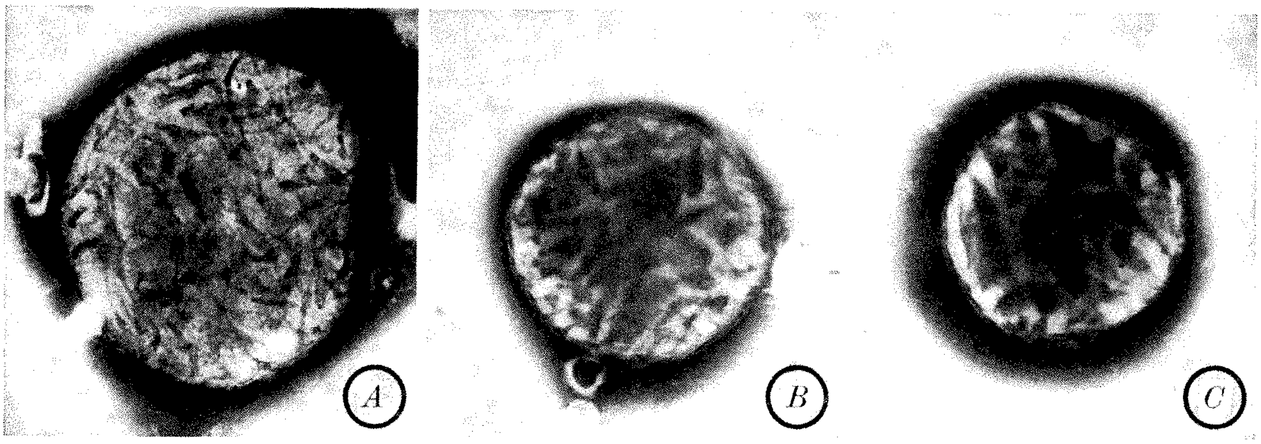

Fig. 2. Isolated rat liver mitochondria in different metabolic states and negatively stained with isotonic ammonium molybdate without preliminary fixation. *A*, Respiratory state 4. The mitochondrion appears as a large sphere embedded in the negative stain which penetrates into the intermembrane space. The inner membrane is superficially infolded. Oxygen uptake rate: low, × 60,000. *B*, Respiratory state 3. The mitochondrial volume is markedly reduced as compared with that of mitochondria in state 4. The inner membrane is deeply infolded. Oxygen uptake rate: high. × 60,000. *C*, Uncoupled respiration. The mitochondrial volume and conformation are similar to the volume and conformation of mitochondria in respiratory state 3. Oxygen uptake rate: high. × 60,000.

25 to 35 per cent diminished with respect to controls (Fig. 1 and Table 1).

Rat liver mitochondria were isolated in 0·25 M sucrose and their respiratory activities monitored with a glass electrode of the Clark type. In our electron microscopy, an improved method of negative staining[9] allowed us to avoid some of the artefacts associated with the fixation of mitochondria. Mitochondria were obtained which retained the relative volumes they have when in suspension, as indicated by a calibration curve of volume changes of mitochondria suspended in sucrose solutions of varying osmolality. The method provided us, moreover, with multiple samples of mitochondria in different respiratory states.

Table 1. EFFECT OF UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION ON THE CONFORMATION AND DIAMETER OF MITOCHONDRIA IN CHICK EMBRYO HEART MYOBLASTS CULTURED *in vitro*

Uncoupling agent	Mitochondrial conformation (per cent)			Mitochondrial mean diameter (μm ± *S.E.*)
	Normal		Condensed	
Control Tyrode	91·9	(1,488)	2·6	0·32 ± 0·003 (320)
100 μM dinitrocresol	3·7	(1,825)	72·3	0·20 ± 0·003 (246)
100 μM dinitrophenol	0	(1,972)	68·4	0·24 ± 0·004 (336)
100 μM pentachlorophenol	8·4	(1,836)	60·2	0·24 ± 0·003 (305)
100 μM dicoumarol	0·1	(2,698)	60·2	0·18 ± 0·002 (490)
1 μm FCCP	14·3	(1,852)	39·6	0·24 ± 0·004 (236)

In parentheses, the number of mitochondria measured.

Mitochondria in respiratory state 4 were expanded spheres (Fig. 2*A*) and the inner sac had a conformation that corresponded to that seen in sections of liver tissue and referred to as "orthodox" conformation[1]. On addition of ADP the respiration rate increased 4–6-fold (state 3), with pyruvate–L-malate as substrate, and the mitochondria exhibited a rapid ultrastructural change: the inner sac contracted, the cristae became deeper and more convoluted and the intracristal spaces increased. The overall organization of the mitochondria thus corresponded to the "condensed" conformation[1]. As a result of this change the total mitochondrial volume decreased from 30 to 40 per cent (Table 2 and Fig. 2*B*). Similar results were obtained by using citrate, α-oxoglutarate or L-glutamate–L-malate as substrates. When 25 μM dinitrophenol was added to mitochondria in respiratory state 4, the rate of oxygen consumption increased to the level of state 3 respiration, but no phosphorylation occurred (Table 2). The uncoupled mitochondria exhibited the same change of ultrastructure and volume as the tightly coupled mitochondria (Table 2 and Fig. 2*C*). Pentachlorophenol (5 μM) elicited similar metabolic and conformational changes in the isolated rat liver mitochondria. The following evidence suggests that our results did not arise from artefacts: three kinds of chemically unrelated uncoupling agents caused similar changes in mitochondria *in vivo*; similar changes in the conformation of the inner sac were observed in mitochondria in living chick embryo heart myoblasts and in isolated rat liver mitochondria. Consistent with this suggestion, and lending further support to the conclusion that both uncoupled and tightly coupled mitochondria undergo conformational changes of a fundamentally similar nature, are the observations of the effects of uncoupling agents on light scattering changes of mitochondria *in vitro*[10]. The conformational changes observed in isolated liver mitochondria were obtained on material which had not been fixed; further, the ammonium molybdate stain does not cause serious metabolic change in phosphorylating mitochondria[11].

In conclusion: (*a*) uncoupling of respiration from phosphorylation in chick embryo heart myoblasts and in isolated rat liver mitochondria is associated with a contracted conformation of the inner mitochondrial sac formed by the inner membrane and matrix; (*b*) the shrinking of the mitochondrial inner sac is accompanied by a reduction in the total volume of the isolated rat liver mitochondria; (*c*) in isolated rat liver mitochondria the changes of conformation and volume seen in uncoupled respiration are indistinguishable from those occurring in respiratory state 3, that is, in coupled respiration.

Table 2. CONTRACTION ASSOCIATED WITH COUPLED AND UNCOUPLED RESPIRATION IN ISOLATED RAT LIVER MITOCHONDRIA

	State 4	State 3	Per cent change	*P*	25 μM DNP	Per cent change	*P*
Oxygen consumption (μl./mg protein/h)	6·2 ± 0·36	24·4 ± 0·48	+394	—	25·0 ± 0·91	+404	—
ADP : O	—	2·51 ± 0·07	—	—	0	—	—
Surface of mitochondria (μm^2)	0·51 ± 0·024	0·30 ± 0·015	−41	< 0·001	0·30 ± 0·016	−41	< 0·001

All results are given as mean ± *S.E.M.* Composition of metabolic medium for polarographic determinations: 10 mM $MgCl_2$; 100 mM NaCl; 10 mM sodium–potassium phosphate buffer, *p*H 7·4; 10 mM tris (hydroxymethyl) aminomethane-HCl buffer, *p*H 7·4; 10·5–14 mM sucrose; substrate, 1·6 mM pyruvate-0·4 mM L-malate; 2·5–4 mg mitochondrial protein per ml.; ADP 2 μmole. Final volume 3·6 ml.; temp. 25° C. 2,4-Dinitrophenol (DNP) was added to mitochondria in state 4. The mitochondria were collected for E.M. after 1 min of state 4 respiration, and after 30 s of state 3 or of uncoupled respiration. For surface determination 75 mitochondria profiles were measured for each of the three groups. Results from four experiments.

These results do not seem to be consistent with the observations of others on uncoupled mitochondria both *in vitro* and *in vivo*[1-5,12], although it should be remembered that Hackenbrock and others studied thin sections. It may be possible to clarify the discrepancies by studies on systems such as isolated mitochondrial preparations whose functioning and ultrastructure can be influenced by a variety of factors. The paucity of the information so far available and the diversity of the results obtained suggest that any generalization on the functional significance of conformational changes in mitochondria in relation to oxidative phosphorylation is at present premature.

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Correlated Changes in Membrane Potential and ATP Concentrations in *Neurospora*

IN a variety of tissues, electrical events seem to be more closely associated with the active transport of ions and with metabolism than with the concentration gradients of ions *per se*. This is evident in the generation of some transepithelial potentials, for example in gastric mucosa[1]; in the hyperpolarization of sodium-loaded (and pumping) nerve and muscle cells[2-4]; and in the rapid depolarization produced by respiratory inhibitors in fungi, algae and higher plant cells[5-7]. Such phenomena may be described in terms of the electrogenic ion pump which separates charges across the cell membrane, thus generating a potential gradient along which ions will move. What is not certain, however, is whether the energy for this particular mode of ion transport is provided by ATP—which clearly drives the neutral coupled pumping of ions in many tissues[8]—or comes more directly from electron transfer, as suggested by Conway's "redox pump" hypothesis[9,10]. We have examined this question for the fungus *Neurospora*, in which the relationship between metabolic and electrical events seems particularly close.

In mature hyphae of this organism the resting membrane potential can be measured with conventional capillary microelectrodes; it is 180–200 mV (inside negative) for hyphae bathed in media containing 10–30 mM potassium, 1 mM calcium and 1–2 per cent glucose or sucrose[11]. The normal intracellular concentration of K^+ is approximately 180 mequiv/kg cell water, so that the diffusion potential for K^+ would be only 45–75 mV. The membrane potential is also rather insensitive to variations of extracellular K^+ or Na^+ concentration, and substitution of these ions by choline, *tris*-(hydroxymethyl(aminomethane, NH_4^+, or Li^+ has little effect. Metabolic inhibitors, by contrast, produce a rapid, large and reversible depolarization (Fig. 1). Within one minute of the addition of 1 mM azide to the external solution, the hyphae depolarize from 200 mV to 25 mV; repolarization on washing out the inhibitor takes place nearly as rapidly, though with a small oscillation before the membrane potential stabilizes at the control value. Separate experiments indicated that there is no significant change of membrane resistance during the rapid depolarization, although it does increase two or three-fold with maintained inhibition[5]. Because of this, we have restricted the present experiments to the period of rapid depolarization, seeking to produce the fastest possible shift of membrane potential with respiratory inhibitors and to determine which metabolic events occur with a comparable time course.

For electrical experiments, mature hyphae are grown on 'Cellophane' sheets resting on a standard medium containing 3 per cent agar[11]; after 20–28 h growth at 25° C, a 'Cellophane' square—with hyphae attached—is cut out and removed to the recording chamber. The chamber is perfused at moderate speeds (15 ml./min, chamber volume 1·7 ml.); control experiments have shown that the local concentration of inhibitor can be brought to any desired value within 0·5–1·0 s if a 20 to 25-fold higher concentration of inhibitor is suddenly introduced into the perfusing fluid. The average results from twenty such experiments, perfusing with 25 mM KCN solution (1 mM cyanide produces a maximal effect), are shown in Fig. 2.

For the first 4–5 s of inhibition, the membrane potential falls somewhat slowly and then declines exponentially. The semilogarithmic plot of the data (inset) at 5 s and beyond is fairly well fitted by a straight line with a slope of − 0·08 log unit/s, or a time constant of 5·4 s.

If the redox pump hypothesis is applicable, inhibition of electron transfer should occur with essentially the same time course as the decline of membrane potential. It is possible to examine this question fairly simply, by measuring the fluorescence of reduced pyridine nucleotides which accumulate during cyanide inhibition or anoxia. A suspension of log-phase cells[12] from a liquid culture is placed in a quartz cuvette, fitted with a pair of entry ports for syringe-needles. A constant stream of fine air bubbles (which keeps the cells suspended and oxygenated)

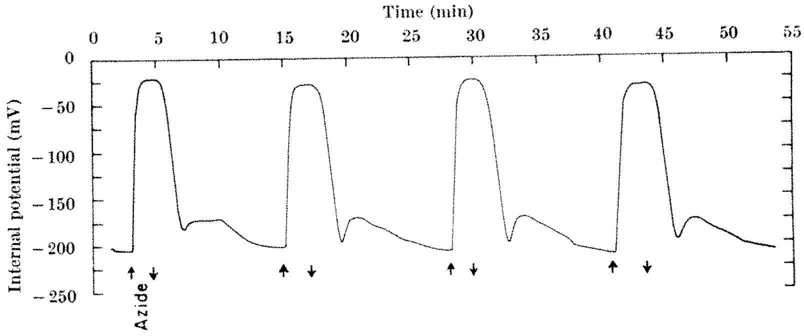
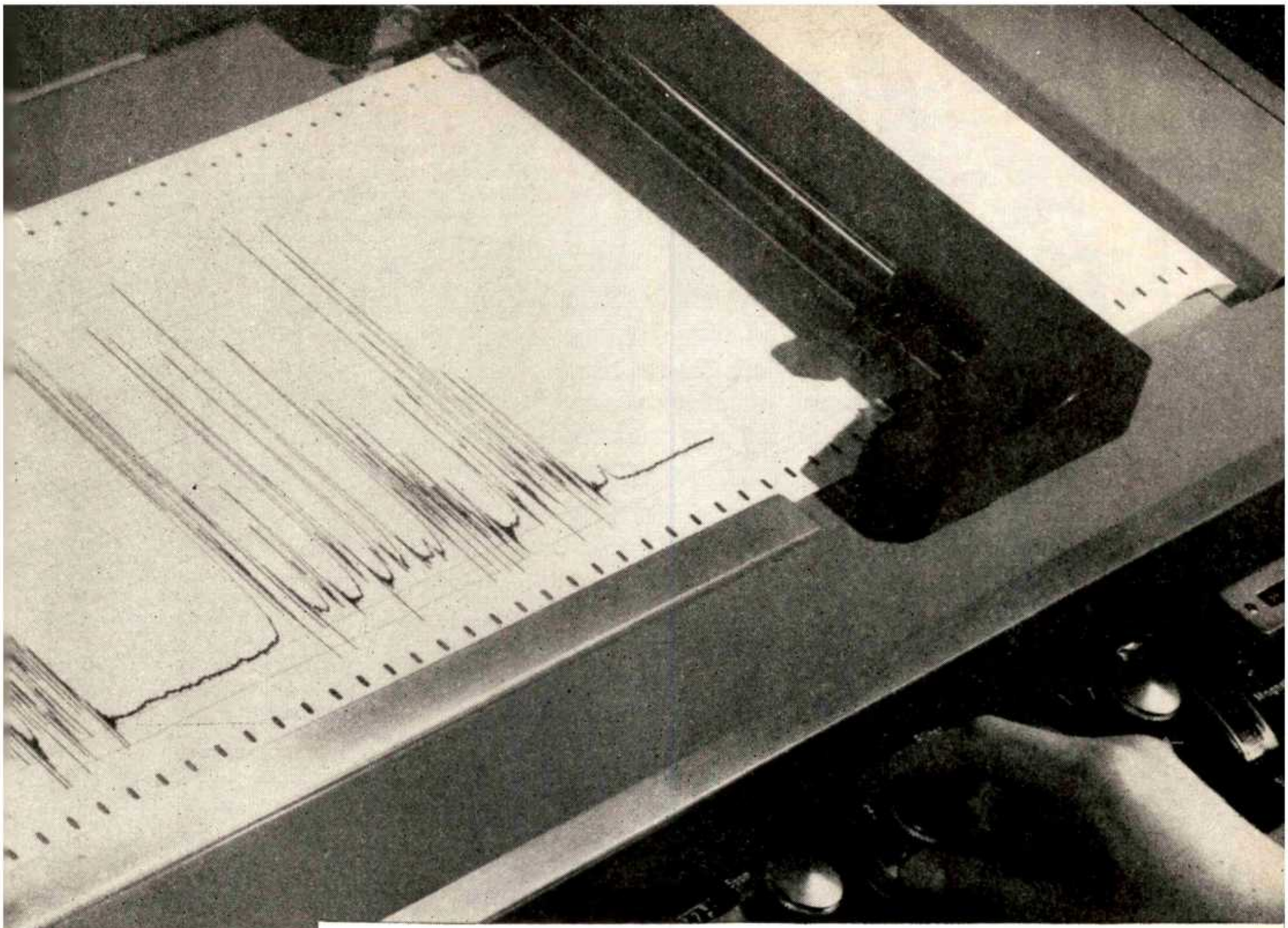


Fig. 1. Effect of respiratory inhibition on the membrane potential of *Neurospora*. Extracellular solution: 10 mM KCl–1 mM $CaCl_2$–2 per cent sucrose, throughout. At the upward arrows 1 mM NaN_3 was added, and bubbling with N_2 commenced. At the downward arrows the nitrogen and azide containing solution was washed out of the chamber.



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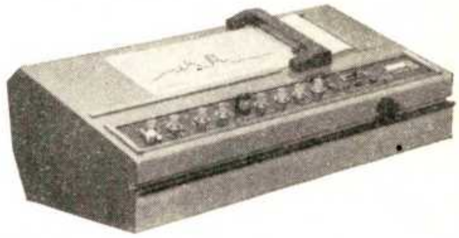

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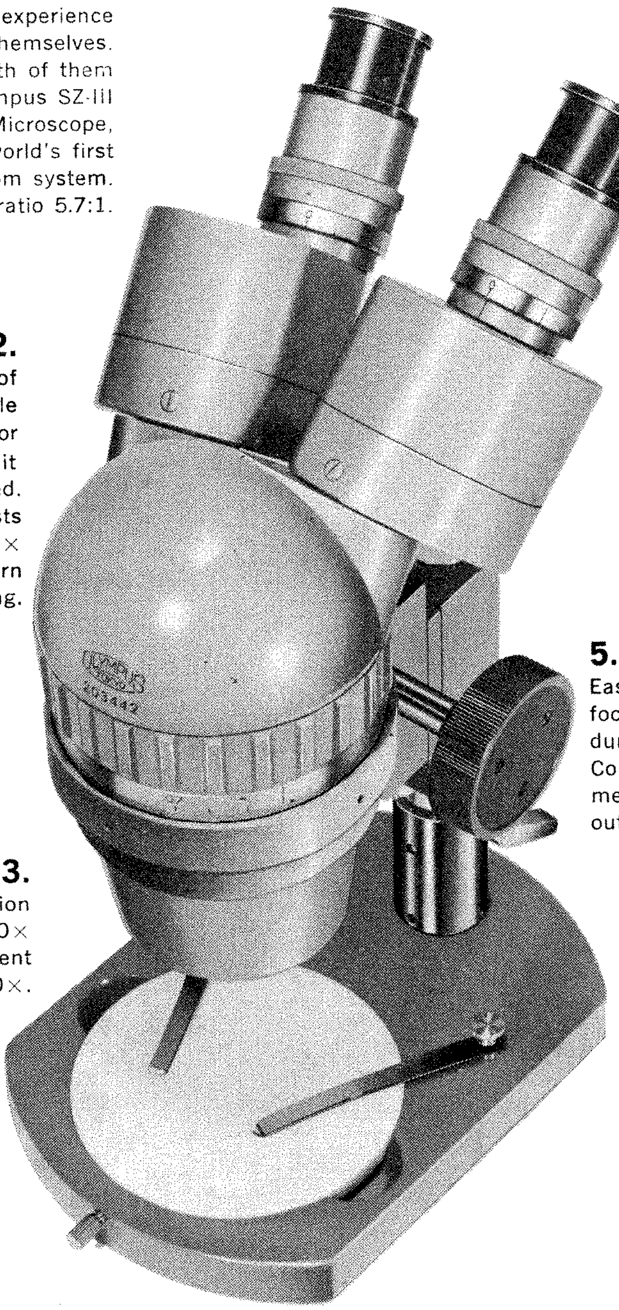

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and a small volume of inhibitor solution can thus be introduced during continuous recording of fluorescence. By adding excess inhibitor to the cell suspension it is possible to achieve a complete respiratory block very rapidly.

The fluorescence record from such an experiment is reproduced in Fig. 3. Because the cell suspension is turbid there is considerable high-frequency noise in the record. But this does not obscure the basic shape of the curve, which fits the sum of two exponentials. The maximal fluorescence increase—signalling maximal reduction of pyridine nucleotides—occurs 8–10 s after injection of cyanide. Partial re-oxidation then follows, perhaps because of adjustments in the glycolytic pathway. Analysis of the fluorescence curve (inset) reveals that primary reduction of pyridine nucleotides takes place with a time constant of 2·6 s, or roughly twice as rapidly as the fastest shift in membrane potential (Fig. 2). Pyridine nucleotides normally occur in great excess over other components of the respiratory electron transfer system, and Chance and Williams[14] have shown that in liver mitochondria they become reduced several-fold more slowly than either cytochromes *a*, *b* and *c*, or the flavoproteins, during the onset of anoxia. If roughly the same relationships hold in *Neurospora*, then Fig. 3 would indicate a time constant of 0·5 s for reduction of the cytochromes and 1·1 s for flavoprotein. It is therefore evident that cessation of electron transfer can occur significantly more rapidly than the decay of membrane voltage in *Neurospora*, so that the two processes can only be coupled indirectly through an energy reservoir.

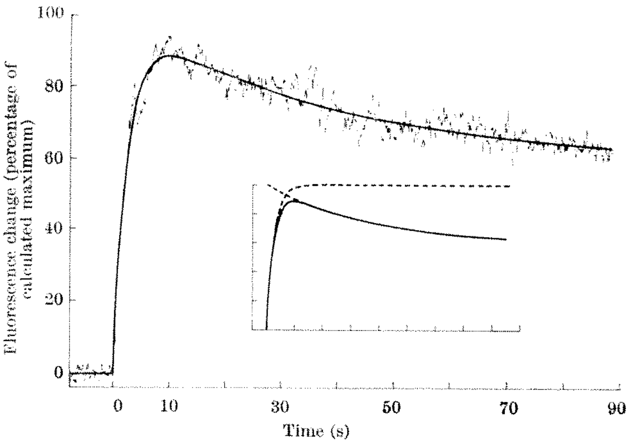

Fig. 3. Fluorescence curve of *Neurospora* during cyanide inhibition. Control medium as in Fig. 2, but with vigorous bubbling by a stream of O_2. Dry weight of cells in 2·5 ml. medium, 6·5 mg. Incident light: 360 nm; emitted light measured at 485 nm; Aminco-Bowman spectrophotofluorometer (American Instrument Co., Silver Spring). At zero time 0·2 ml. of 1 M KCN (*p*H 5·8) was injected. The smooth curve is drawn according to the equation:

$$y = 100\,(1 - e^{-t/2.67}) - 41{\cdot}4\,(1 - e^{-t/39.0})$$

where the specific numbers were obtained by a least-squares fitting procedure[13]. Inset: separation of the two exponential components.

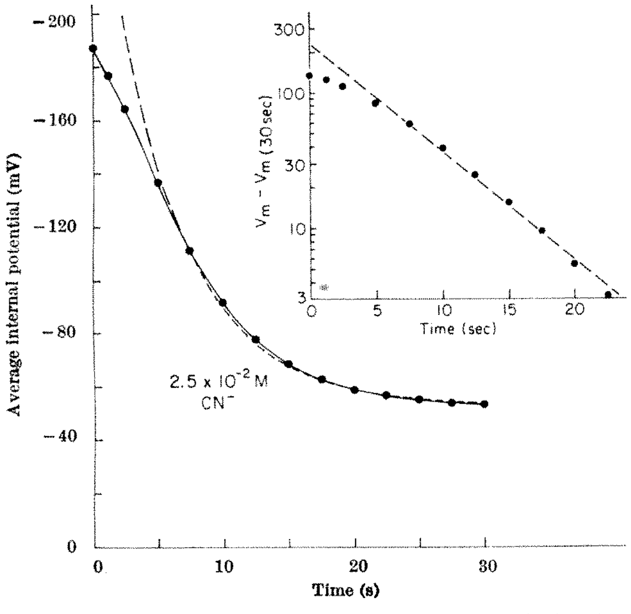

Fig. 2. Time-course of the voltage decay at the onset of cyanide inhibition. Control medium: 20 mM dimethylglutaric acid (DMG, an inert buffer; *p*H 5·8)—25 mM KOH—1 per cent glucose. At zero time, a solution of 20 mM DMG (*p*H 5·8)–25 mM KCN–1 per cent glucose was introduced. All points and the solid curve represent average membrane potentials from twenty trials on thirteen different hyphae. *S.E.* at all points was less than 6 mV. Dashed curve, obtained from Fig. 4, has a time constant of 5·4 s. Inset: semilogarithmic plot.

That this reservoir could be ATP is indicated by studies of intracellular ATP levels at the onset of cyanide or azide inhibition. A well-aerated suspension of liquid-cultured cells is harvested on a sintered glass suction filter; in the conditions used, removal of the solution (20 ml.) takes about 2 s. Just before the liquid is gone, fresh solution containing inhibitor is poured on to the cells and sucked through. At the appropriate time the cell mat is placed in petroleum ether at −70° C. By this method frozen cell mats can be obtained 3–4 s after the addition of inhibitor. The frozen mats are dried under vacuum at −20° C, weighed, pulverized and extracted with perchloric acid. The neutralized extract is assayed for ATP using the firefly luciferin–luciferase technique[15].

There is no significant difference between the effects produced by 10^{-3}, 10^{-2} (Fig. 4) and $2{\cdot}5 \times 10^{-2}$ M cyanide (not shown). Within 30 s the calculated intracellular ATP concentration, $[ATP]_i$, falls from 2·7 mM to 0·25 mM. The semilogarithmic plot of the same data (inset) gives a straight line with a slope of −0·08 log unit/s, or a time constant of 5·4 s. ATP is broken down rapidly by *Neurospora* once respiration is blocked, and this may be attributed to three factors. (1) The organism is unable to derive appreciable amounts of energy from glycolysis alone; it cannot grow in the absence of oxygen[16]. (2) It does not store energy-reserve compounds such as creatine phosphate or arginine phosphate. (3) Its normal steady-state oxygen consumption is very high, 50–75 *ul.* O_2/mg dry wt./h[5], which corresponds to approximately 0·3 mmoles/kg cell water/s (1 mg dry = 2·54 mg cell water). The P/O_2 ratio in *Neurospora* is 4 rather than 6 (E. Smith and C. W. Slayman, manuscript in preparation), apparently because this organism, like yeast[17], lacks "site I" phosphorylation. Thus the steady-state turnover of high-energy phosphate must be approximately 1·2 mmoles/kg cell water per s. The hydrolysis of two phosphate bonds per molecule in the ultimate breakdown of ATP to AMP, with a time constant of 5·4 s, corresponds to a hydrolytic rate of 0·9 mmoles/kg cell water per second—close to the calculated steady-state turnover.

The curve obtained in Fig. 4 has been superimposed (dashed lines) on the electrical data in Fig. 2 to demonstrate that after 4–5 s of inhibition the membrane potential declines in parallel with $[ATP]_i$. In other words, membrane voltage (and presumably the pumped ionic current) seems to be linearly proportional to $[ATP]_i$ at levels below 1·5 mM. At higher ATP concentrations, however, the voltage appears to saturate, so that a plot of the membrane potential against $[ATP]_i$ yields a rectangular hyperbola, as might be expected for an enzymatic process.

To a certain extent the result in *Neurospora* resembles those obtained by Durbin[18] and by Forte, Adams, and Davies[19] for gastric mucosa, where a linear relationship seems to exist between tissue ATP levels and the short-circuit current or rate of secretion of H^+. The response to respiratory inhibitors in *Neurospora* is, however, both much faster and much less complicated by cellular

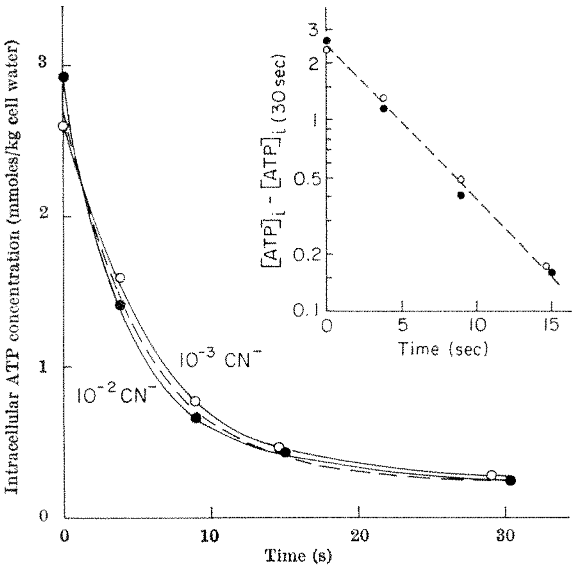

Fig. 4. Decay of intracellular ATP at the onset of cyanide inhibition. Control medium as in Fig. 2. At −2 s the cell suspension was filtered, and at zero time a buffer solution containing 1 mM (○—○) or 10 mM (●—●) KCN (*p*H 5·8) was sucked through the cell mat. Dashed curve: replot of the least-squares line from the inset. Inset: semilogarithmic plot; least-squares slope: −0·080±0·002 log unit/s; time constant: 5·4±0·2 s.

heterogeneity than in a complex tissue. On the other hand, direct measurements of ion-pump activity—like those by the short-circuit technique—are more difficult with this microorganism than with large pieces of tissue. Because we have not measured short-circuit currents and have not really controlled $[ATP]_i$ of the hyphae at will, it would be premature to conclude that ATP is in fact the direct energy source for the membrane potential. But clearly the fluorescence data require some kind of intermediary between electron transfer and the membrane potential, and the nearly exact coincidence between the rates of decay of ATP and the voltage would point to ATP.

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Trypan Blue-induced Deletions in Duplication Strains of *Aspergillus nidulans*

IN the filamentous fungus *Aspergillus nidulans*, strains with a chromosome segment in duplicate (one in normal position, one translocated to another linkage group) are unstable at mitosis[1,2]. This "mitotic non-conformity" is provoked by chromosomal imbalance and initial changes of genotype are confined largely to the segments carried in duplicate[3,4]. Duplication strains, which have a characteristic morphology and reduced linear growth rate, give sectors produced by nuclei which have undergone spontaneous deletions, of variable size, from one or other duplicate segment. Sectors can almost always be scored unequivocally by their improved morphology and relative growth advantage, determined by nuclei more nearly haploid in quantity than those of the duplication parent. In suitably marked strains, loss of one or more dominant alleles gives sectors differing from the parent in conidial colour and/or nutritional requirements (Fig. 1*a*). The approximate size of each deletion, and the segment involved, can be determined by genetic analysis or sometimes

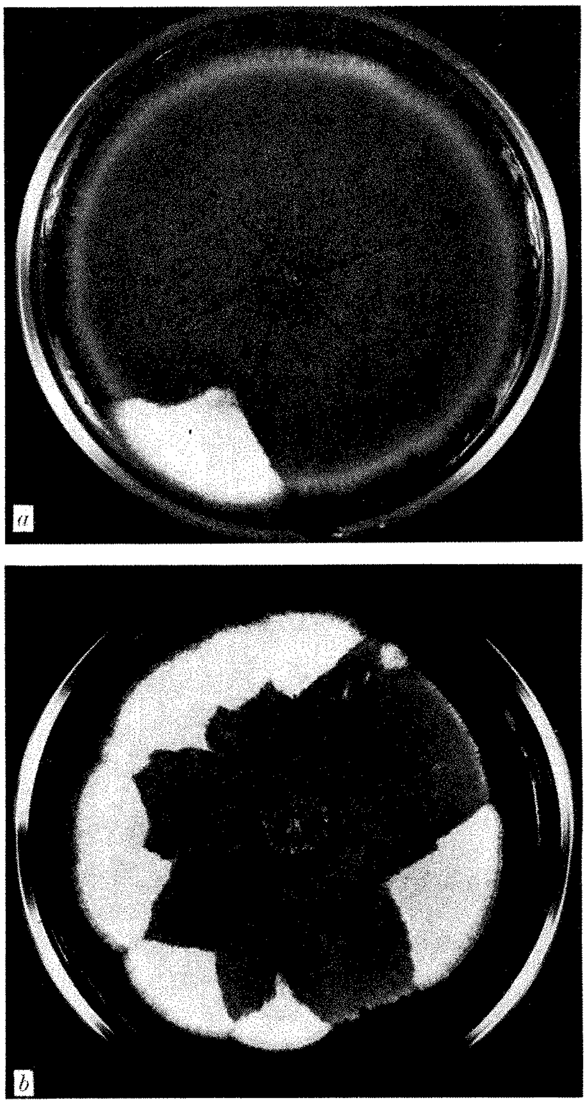

Fig. 1. Yellow sectors, produced by a duplication strain with green conidia, following deletion of the dominant y^+ allele: *a*, control after 7 days; *b*, grown on 300 μg/ml. trypan blue for 9 days.

simply by the morphology, nutritional requirements and stability or instability of the sector carrying it. This system seems to offer a simple screening test for deletion-producing agents and for any selective activity they might show towards particular chromosome regions. In addition, success in modifying sector frequency might help in elucidating the processes of mitotic non-conformity. The first attempt to induce deletions, using trypan blue (TB), has been successful.

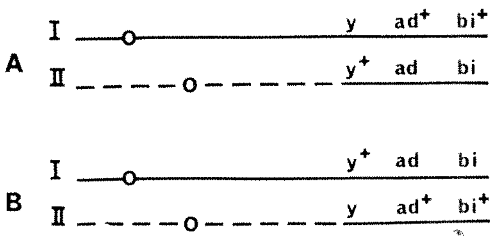

Fig. 2. Genotypes of strains used. Linkage groups I and II are shown by unbroken and broken lines respectively. The recessive, mutant alleles determined: *y*, yellow conidia, as opposed to wild-type green; *ad* and *bi*, requirement for adenine and biotin respectively.

Conidia of strain A (Fig. 2) were inoculated at the centre of 9 cm dishes of complete medium[5] containing 0 to 300 μg/ml. TB; sectors were scored after 7 days at 36° C. Plated conidia showed 100 per cent viability on all the TB concentrations used, but linear growth was significantly reduced above 50 μg/ml. ($\bar{x}_c = 7{\cdot}25$, $\bar{x}_{50\ \mu g} = 7{\cdot}01$, $t_{(70)} = 4{\cdot}0^{***}$) and was 71 per cent of the control at 300 μg/ml. ($\bar{x}_{300\ \mu g} = 5{\cdot}18$, $t_{(70)} = 41{\cdot}4^{***}$). The number of sectors per colony showed a Poisson distribution both in controls and all treatments. The Kolmogorov–Smirnov two-sample test was therefore used for all comparisons. There were slightly more yellow (20) than green (14) sectors in the forty control dishes. Although the difference was not significant in this one control series, it was consistent with general experience of the system and showed that deletions which included the y^+ allele, on the translocated segment, exceeded the sum of all other deletions. This excess of yellow sectors was generally maintained at all levels of treatment and, while TB significantly raised the frequency of yellow sectors at concentrations in excess of 50 μg/ml., only a marginal increase in the frequency of green sectors was produced at 300 μg/ml. (Table 1). Mutagenic activity at the higher TB concentrations was underestimated as a result of growth inhibition, which reduced the number of nuclei exposed to test, and by the large number of sectors which drastically reduced the proportion of the colony periphery available for further sectors. In fact, colonies grown for 9 days on 300 μg/ml. TB had a largely yellow periphery (Fig. 1*b*). This, and the dose response results, implied a preferential activity of TB on the segment carrying y^+.

Table 1. SECTORS PRODUCED BY CONTROL AND TRYPAN BLUE-TREATED COLONIES OF STRAIN *A*

TB treatment (μg/ml.)	No. of dishes	Mean No. of yellow sectors	Significance of comparison with control	Mean No. of green sectors	Significance of comparison with control
Control	40	0·50		0·35	
0·025	40	0·38	—	0·55	—
0·25	40	0·53	—	0·43	—
2·5	40	0·45	—	0·38	—
50	32	1·44	5–1 per cent	0·47	—
300	32	4·34	< 0·1 per cent	1·03	10–5 per cent

Strain B (Fig. 2), with mutant and wild-type alleles reversed between the duplicate segments, gave few spontaneous or induced yellow sectors, though it responded as sensitively to TB as did strain A. This showed that the selective activity of TB on the translocated segment of strain A was determined not by the alleles it carried but by its structural position in the genome. Many induced sectors retained some degree of the duplication morphology; they arose from only a partial deletion, preferentially from the translocated segment. The preponderance of induced yellow sectors from A and green from B could not be explained in terms of a possible advantage of normal haploid nuclei, resulting from total deletion of the translocated segment, over translocated haploids resulting from the alternative deletion. Further characterization of the sectors and an extended statistical analysis will be published later.

The translocated state of one of the homologous, duplicate segments must account for its greater susceptibility to spontaneous deletion; this can be explained tentatively in terms of a greater liability to replication error[3]. It is less easy to offer a plausible explanation for the differential action of a mutagen. Tests being made with other mutagens should show whether the differential action reflects a special property of trypan blue or whether it results frequently from the interaction of mutagens and the processes of mitotic non-conformity.

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Cell Wall Thickness, Size Distribution, Refractive Index Ratio and Dry Weight Content of Living Bacteria (*Staphylococcus aureus*)

I HAVE described[1] experiments on the identification of living bacteria using their differential light scattering signatures, following a theoretical study[2] which proposed that different biochemical structures should scatter light in essentially different manners. For water suspended bacteria, the Rayleigh–Gans scattering theory[2] does indeed describe the light scattering measurements to within experimental error[3]. As a consequence of the excellent correspondence between theory and experiment, I have been able to estimate the average cell wall thickness of *Staphylococcus aureus* from light scattering data obtained from living cells suspended in water. Further, I have deduced a rather accurate characterization of the cellular size distribution, as well as a certain refractive index ratio. On the basis of the ratio the dry weight content of the cell wall and cytoplasm can be estimated. Until now there seems to have been no means[4] by which the ultrastructure of living cells might be measured directly.

Fig. 1 presents the recorded differential scattered intensity of vertically polarized 632·8 nm radiation from a viable culture of *S. aureus* (supplied and identified by Mrs Peggy Flynn, Goleta Valley Community Hospital Laboratory). The cells were grown at 37° C for six hours on heart infusion agar and then suspended in double distilled water. Under phase contrast examination they appeared spherical and predominantly single. The upper curve of Fig. 1 is approximately a five-fold amplification of the lower curve. The recordings in Fig. 1 are particularly well defined. The very deep first minimum is

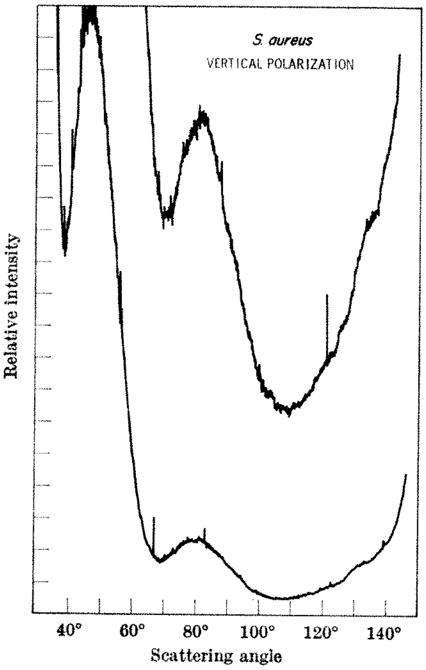

Fig. 1. Relative differential scattered intensity of vertically polarized 632·8 nm light from *S. aureus* grown on heart infusion agar at 37° C for six hours and resuspended in water. The recordings are uncorrected.

characteristic of the presence of a very narrow size distribution of cells and the standard deviation of the radius amounts to less than 20 per cent.

Fig. 2 presents a recording of the differential scattered intensity from the same cells, but illuminated by horizontally polarized radiation at 632·8 nm. Two features are of particular significance: the near vanishing of the signal at a scattering angle of 90° and the appearance of only one peak. All objects whose scattering is described by Rayleigh–Gans theory produce a scattering pattern characterized by a $\cos^2\theta$ factor for horizontally polarized radiation. The same factor is responsible for the vanishing of the second peak shown in Fig. 2, because the data can be obtained immediately by multiplying the data from Fig. 1 by $\cos^2\theta$. In other words, we learn nothing new by performing horizontally polarized light scattering measurements on Rayleigh–Gans scatterers. Thus, irrespective of the size, shape, structure or size distribution of an ensemble of Rayleigh–Gans scatterers, the intensity at 90° should always be zero. The slight positive recording shown at 90° in Fig. 2 arises from the detector's lack of perfect angular resolution. (The resolution of the detector is 2°.)

The solid curve of Fig. 3 presents the data of Fig. 1 corrected for the $\sin\theta$-foreshortening of the effective scattering volume[1,5] as seen by the detector. The two dashed curves characterize good and poor theoretical fits, respectively. Discrepancies between theory and experiment are discussed later.

The cells present in the scattering ensemble were found to be well characterized by a size variation given by

$$N(\rho) = N_0 \exp\left[-(\rho-\rho_0)^2/\Delta^2\right] \qquad (1)$$

where $\rho = kr$, r is the radius of a cell, $k = 2\pi n_0/\lambda$, n_0 is the refractive index of water at the wavelength λ of the incident radiation, N_0 is a normalization constant, Δ is the deviation in ρ about the average value ρ_0 and $N(\rho)$ is the number of cells between ρ and $\rho + d\rho$. The quantities ρ_0 and Δ must be determined experimentally. The distribution of equation (1) is quite similar to the volumetric distribution measured by Harvey and Marr[6] using a Coulter counter. A rectangular distribution has also been examined and yields essentially identical results. Indeed, for the narrow size distribution found in the present study, the exact shape chosen to characterize it is immaterial.

The data of Fig. 3 cannot be accounted for on the assumption that the cells are homogeneous or characterized by some average dielectric constant. Examination by electron microscope shows that the cells are predominantly inhomogeneous, spherically symmetric objects having a well defined cell wall. A simple model of the cells was chosen to characterize this basic type of structure, namely a spherical cell of radius r and cell wall thickness t; the refractive index of the cell wall relative to the suspending media was m_1 and that of the cytoplasm m_2. Because a distribution of sizes was present in the scattering ensemble, the fractional radius f of the cytoplasm was a useful parameter, that is, $f = (1 - t/a)$. In the Rayleigh–Gans approximation the relative intensity scattered by an ensemble of such cells is given by

$$I(\theta) = \int_0^\infty N_0 \exp\left[-(\rho-\rho_0)^2/\Delta^2\right] i(\theta,\rho)\,d\rho \qquad (2)$$

where

$$i(\theta,\rho) = [k^3 V(m_1-1)/(2\pi)]^2\,[G(u) + \beta f^3 G(fu)]^2$$

$$u = 2\rho\sin\tfrac{1}{2}\theta, \qquad \beta = (m_2 - m_1)/(m_1 - 1)$$

$$G(u) = (3/u^3)\,(\sin u - u\cos u)$$

and

$$V = \frac{4}{3}\,\pi r^3$$

A weighted least-squares fit of equation (2) to the corrected data of Fig. 3 was completed with a high-speed digital computer (CDC 6,400). The auxiliary conditions that the first four extrema occur at the experimentally measured locations (to within ±1°) were also imposed by the computer program. On this basis, the best values of the four parameters were found to be

$$\rho_0 = 6{\cdot}3 \pm 0{\cdot}3$$

$$f = 0{\cdot}82 \pm 0{\cdot}04$$

$$\Delta = 1{\cdot}05 \pm 0{\cdot}1$$

$$\beta = -0{\cdot}60 \pm 0{\cdot}15$$

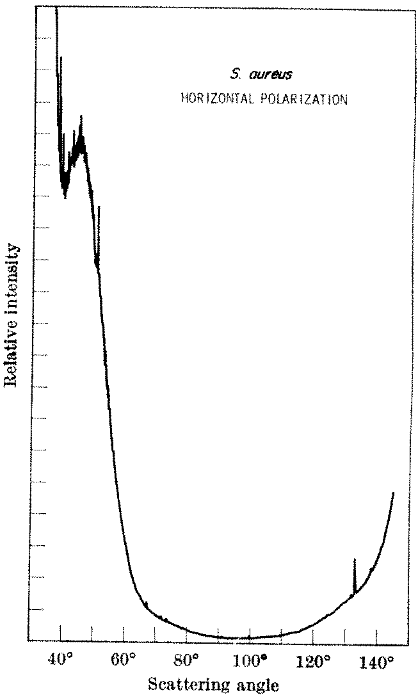

Fig. 2. Relative differential scattered intensity of horizontally polarized 632·8 nm light from the same *S. aureus* suspension as in Fig. 1.

The uncertainties in the above figures were established during the extensive numerical analysis. Both of the dashed curves of Fig. 3 have extrema at the experimentally measured positions, and both were calculated on the basis of equation (2), normalized at 47·5°. The parameters for these typical "good" and "poor" fit curves were, respectively, $kr = 6{\cdot}3$, $5{\cdot}6$; $\Delta = 1{\cdot}05$, $1{\cdot}40$; $\beta = -0{\cdot}58$, $-0{\cdot}80$; and $f = 0{\cdot}82$, $0{\cdot}84$. The slight discrepancies between the corrected data and "good" fit curves of Fig. 3 could be eliminated by more extensive numerical and theoretical analyses in which averages would be taken over the 2° angular resolution of the detector system and use would be made of the small contributions of internal reflexions at the glass interfaces of the cuvette[2,5] (Fresnel factors), the effects of cellular agglomerates (about 10 per cent of the cells were observed to be doubles) and the effects of internal structural features such as septum formation in a small fraction of the population. These corrections are unimportant here.

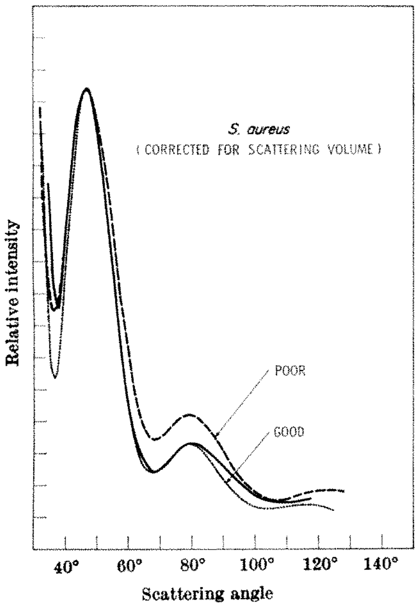

Fig. 3. The relative differential scattered intensity (solid curve) corresponding to the data of Fig. 1, but corrected for instrumentation foreshortening. The two dashed curves correspond to "good" and "poor" theoretical fits.

Specific values of cell radius, cell wall thickness and size distribution can be obtained from the above parameters. $\lambda = 632{\cdot}8$ nm and $n_0 = 1{\cdot}33$ at this wavelength, so that we have immediately

$$\text{(average radius) } r_0 = \rho_0/k = \rho_0\lambda/(2\pi n_0) = 477 \text{ nm}$$

$$\text{(average thickness) } t = (1-f)r_0 = 0{\cdot}18 \times 477 = 86 \text{ nm}$$

$$\text{(radius deviation) } \Delta/k = 79{\cdot}6 \text{ nm}$$

Electron micrographs prepared by Professor James Cronshaw suggest that for *S. aureus* grown in similar conditions the cell-wall thickness is about 18 nm and the average cell radius is about 440 nm. Salton[4] quotes a range of 10–20 nm as typical for the cell-wall thickness of *S. aureus* as deduced from electron micrographs. Although Knaysi[7] agrees in general with Salton's estimates, he does point out that ". . . in the living state [the cell wall] . . . thickness is probably several times greater. . . ." His conjecture has thus been shown to be very good. The larger cell and cell wall sizes I have deduced are consistent with the belief that cells shrink during fixation, much of the shrinkage apparently occurring in the cell wall itself.

If the cytoplasm of the cells were of the same refractive index as water, the ratio factor β would be -1. But β is greater than this value and the refractive index of the cytoplasm is, as expected, greater than 1·33. Unfortunately the refractive indices of the cell wall and cytoplasm are intimately related in the Rayleigh–Gans theory and only the ratio β may be directly determined from relative differential scattered intensity measurements. Absolute intensity measurements are particularly difficult to make, for not only must the cellular concentrations be accurately determined, but extensive calibration procedures must be introduced[5] as well.

It is not surprising that the refractive index ratio β has the greatest experimental uncertainty because, for homogeneous objects, the Rayleigh–Gans theory is essentially insensitive to scatterer refractive index. Nevertheless, if the average refractive index of the cells could be measured by another method, the data produced here might be useful in determining the refractive indices and perhaps the dry weight fractions for both cell wall and cytoplasm[8]. If, for example, the average refractive index values deduced from immersion refractometry for *B. cereus* and *B. megaterium*[9] were reasonably similar to those of *S. aureus*, the latter species would have a cytoplasm of refractive index (at 632·8 nm) 1·365 and a cell wall of index 1·42. Barer's result that the mean refractive increment is proportional to the dry weight would yield a cell wall structure consisting of about 50 g/100 ml. solid content, whereas the cytoplasm would consist of only 19·5 g/100 ml. These figures are tentative because of the large uncertainties associated with both my refractive index data and those of ref. 9; but the results are consistent and reasonable.

Work in progress on the scattering characteristics of single viable cells is expected to contribute data about the ultrastructure and chemical composition of bacteria.

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Evidence for Polygenic Control of Plasmodial Fusion in *Physarum polycephalum*

THE vegetative phase of Myxomycetes (true slime moulds) is a macroscopic, multinucleate cell called a plasmodium. In many species, this structure characteristically has the capacity to migrate over solid surfaces, a feature which readily lends itself to the study of somatic compatibility. For example, when two plasmodia collide they may fuse or fail to do so, depending on whether or not they are genetically similar at certain loci[1-5]. In the two species studied so far, two somewhat different explanations have been offered. For *Didymium iridis*, a polygenic system with simple dominance at each locus has been proposed[1-3], while a one locus, multiple allelic system with no dominance has been suggested for *Physarum polycephalum*[4,5].

We have evidence that in our isolate of *P. polycephalum*, plasmodial fusion is apparently determined polygenically, as in *D. iridis*.

We used material designated Pp11, obtained from Iowa State University. The original plasmodial specimen was grown according to Camp's method[6], and within a week numerous sporangia developed. In myxomycetes, the diploid plasmodial nuclei undergo meiosis during sporangial formation, and each mature spore possesses a single haploid nucleus. When a spore germinates, it gives rise to a single uninucleate, haploid amoeba which, if isolated, can give rise to a clonal population of several million cells. Twenty-one clones were obtained and subsequently crossed with sibling testers to determine their mating types. Twelve were found to be of one mating type and seven of the other, while two could not be classified with certainty. No attempt was made to determine whether these mating types were duplicates of those described by Dee[7]. Fertile crosses made on half strength corn meal agar yielded macroscopic plasmodia in 5–10 days. These were fed sterile 'Quaker' oats until they covered most of the agar surface in 15 × 100 mm Petri dishes. Transfers and new feedings were repeated at weekly intervals.

To test for compatibility, blocks of agar containing vigorous pieces of plasmodia from two different crosses were placed side by side on the surface of 2 per cent water agar. When the plasmodia collided they were examined with a dissecting microscope, using sub-stage lighting and magnifications up to × 120. Scoring for fusion or non-fusion has been described and illustrated in detail elsewhere[1,3,4].

Table 1. SEVEN PLASMODIA TESTED FOR FUSION IN ALL DIFFERENT PAIRED COMBINATIONS

	3·5	1·3	P	5·11	11·21	1·17	1·4
3·5		–	–	+	–	–	–
1·3			–	–	–	–	–
P				–	–	+	–
5·11					–	–	–
11·21						–	+
1·17							–
1·4							

+ Fusion; –, non-fusion. All tests were made at least twice.

Results were obtained by testing the original parental plasmodium obtained from a sclerotium, and ten plasmodia derived from clonal crosses. In one experiment, seven of these plasmodia were tested in all combinations, taking two at a time (Table 1). P indicates parental, whereas two numbers separated by a dot indicate plasmodia obtained by crossing two F_1 clones; that is, clones 1 × 3. The pattern of fusion and non-fusion shown in Table 1 makes it possible to assign these plasmodia to four classes: (*a*) 3·5 and 5·11; (*b*) 1·3; (*c*) 1·17 and P; (*d*) 1·4 and 11·21. These data are not consistent with a one locus, multiple allelic compatibility system. Based on the one locus hypothesis, the parental plasmodium could carry no more than two fusion alleles; for example, F_1 F_1, F_2 F_2 or F_1 F_2. Our results show that 1·3, 1·4 and 1·17 belong to three different classes. Assuming that clone 1 carries a given allele, F_1, then clones 3, 4 and 17 could carry among them, at most, the F_1 and F_2 alleles.

In another experiment, four plasmodia (1·3, 1·4, 1·17 and 3·5) were used as testers to determine whether four additional F_1 plasmodia (11·25, 11·26, 17·18 and 5·17) belonged to new fusion classes (Table 2). These results show that only one of these, 5·17, corresponds to an established class. None of the remaining three fused when tested in all possible pairwise combinations. Thus the ten F_1 plasmodia tested were distributed among seven fusion classes. These data argue strongly against a one locus control system. According to this hypothesis, fusion occurs between plasmodia which have identical genotypes at the plasmodial compatibility locus[4,5]. For example, according to the one locus hypothesis, from the fusion of 1·17 and 5·17, we would conclude that clones 1 and 5 are identical at the compatibility locus. Accordingly the fusion of 3·5 and 5·11 indicates that clones 3 and 11 are identical at this locus. If the compatibility genotypes of clones 1 and 5 are identical, and so are those of clones 3 and 11, then fusion should occur between 1·3 and 5·11; however, no fusion was observed. These results cannot be explained by the one locus hypothesis. Furthermore, the fusion of 1·17 and 5·17 and the failure of 1·3 and 3·5 to fuse indicate the existence of dominance for at least one fusion locus.

Table 2. FOUR TESTERS (1·3, 1·4, 1·17 and 3·5) PAIRED IN ALL COMBINATIONS WITH FOUR F_1 PLASMODIA

	11·25	11·26	17·18	5·17
1·3	–	–	–	–
1·4	–	–	–	–
1·17	–	–	–	+
3·5	–	–	–	–

+, Fusion; –, non-fusion. All tests were made at least twice.

It is obvious that the myxomycetes provide excellent material for the study of the genetic control of somatic compatibility. These preliminary data about *P. polycephalum*, an organism used in many kinds of research[8,9], will be followed soon by a more extensive account. This is part of an expanding programme of study of plasmodial compatibility[1–3,10] started several years ago[11]. It will be interesting to find out whether various species have different genetic systems for the control of fusion, and whether different isolates of the same species vary in this characteristic. Recent work[4,5] on *P. polycephalum*, when compared with that described here, suggests strain differences. Alternatively, it should be pointed out that earlier data about this species fit a polygenic system almost as well as a one locus system.

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Plant Protoplasts for Use in Somatic Cell Hybridization

SOMATIC cell hybridization is a potentially useful technique for the introduction of genetic variability into plant species. One proposed method involves fusion of plant protoplasts (which is a cell lacking its wall—defined here on the basis of light microscope examination) to form a hybrid cell which can be induced to form a plant[1]. Plant protoplasts produced by the use of crude, commercial lytic enzymes are not always healthy (as defined by consideration of factors such as cytoplasmic streaming, presence of cytoplasmic strands and position of the nucleus within the cell, in relation to survival time)[2,3]. Peroxidase[2], basic proteins[3] and ribonuclease[4] have been implicated as possible deleterious agents. The use of purified enzymes offers a more rigorously defined system for obtaining healthy protoplasts. We have used purified preparations of cellulase (EC 3.2.1.4), pectate lyase (EC 4.2.99.3) and endopolygalacturonase (EC 3.2.1.15) to prepare protoplasts from a range of plant cell suspension cultures. We have also prepared inducible streptomycete enzymes lytic towards plant cell walls.

Plant suspension cultures were grown in defined conditions[5]. Semi-purified pectinases included pectate lyase prepared from 'Pectinol 59L' (Rohm and Haas, Philadelphia)[6] and endopolygalacturonase from *Saccharomyces fragilis* C351[7]. We obtained electrophoretically purified *Trichoderma viride* hydrocellulase with high C_1 activity[8] from Miles Laboratories, Elkhart, Indiana. Polyacrylamide gel electrophoresis at *p*H 8·9 showed that each final enzyme preparation had a major and several minor components. The enzymes were sterilized by filtration and used in the concentrations given in Table 1. The final concentration of sorbitol in all experiments was 0·35 M. Protoplasts were produced as follows. Cells were washed and resuspended in 0·01 M sodium citrate (*p*H 5·5) containing 0·35 M sorbitol. The cell suspension (10 µl.) was then incubated with glycosidases in hanging drops (40 µl.) using depression slides. Mineral oil around the coverslip prevented evaporation. Microscopic observations were made during 24 h. Hydrocellulase alone forms protoplasts from *Glycine max* L., *Meliotus alba* Desr. variety 'Arctic' and *Linum usitatissimum* L. while pectinases had no further effect (Table 1)—when they were added the resulting protoplasts were less healthy. This was probably because of the toxic nature of extraneous substances in the semi-purified pectinases. Power and Cocking[9] obtained excellent yields of protoplasts when they used mixtures of crude commercial cellulases and pectinases against tobacco leaves.

Table 1. PROTOPLAST FORMATION USING COMBINATIONS OF HYDROCELLULASE AND PECTINASE

Cell cultures	Hydro-cellulase*	Hydro-cellulase + pectate lyase †	Hydro-cellulase + endopoly-galacturonase ‡	Hydrocellulase + pectate lyase + endopoly-galacturonase
Glycine max L.	+	+	+	+
Rosa sp. variety 'Sceptre'	–	–	–	–
Triticum monococcum L.	–	–	–	–
Haplopappus gracilis (Nutz) Gray	–	–	–	–
Meliotus alba Desr. variety "Arctic"	+ +	+ +	+ +	+ +
Linum usitatissimum L.	+	+	+	+

Culture conditions are given in ref. 5. Negative results were obtained using endopolygalacturonase and pectate lyase either singly or in combination.

* Final concentration was 0·026 U/40 µl. (A unit is defined as the release of 1 µmole of monosaccharide equivalent per minute from carboxymethyl cellulose[2], pectin[3] and polygalacturonic acid[4] respectively.)

† Final concentration was 0·21 U/40 µl.

‡ Final concentration was 0·42 U/40 µl.

+ +, 5–20 per cent protoplasts in 3 h.

+, 5 per cent protoplasts in 3 h.

–, No protoplasts in 24 h.

Cell walls of the plant cultures were prepared by grinding cells with glass beads (120 µm) in an ice-cooled 'Omnimixer' at high speed. Crude wall preparations were boiled, treated with purified α-amylase for 2 days in the presence of toluene, separated from the glass beads and fine particulate materials by differential centrifugation and then washed exhaustively with water and freeze dried. The gross sugar composition was determined by analysing the products of acid hydrolysis by paper chromatography[5]. Pectin hydrolysates were chromatographed using ethyl acetate–pyridine–acetic acid–water (5 : 5 : 1 : 3) and identified with naphthoresorcinol/phosphoric acid reagent[10]. Composite results are presented in Table 2. Cell walls of each species contained substantial proportions of cellulose and pectin. *Haplopappus gracilis* (Nutz) Gray, *Rosa* sp. var. 'Sceptre' and *Triticum monococcum* L. also had substantial amounts of arabinose, galactose and xylose. There is a fair degree of correlation between protoplast forming ability (Table 1) and cell wall composition (Table 2)—cellulase on its own formed protoplasts from species with cell walls containing the least amounts of arabinose, galactose and xylose. There were, however, greater amounts of alkali soluble polysaccharides in *M. alba* cell (51 per cent) than in *H. gracilis* cell walls (26 per cent). The relative amount of cellulase used (final concentration 0·058 per cent) (Table 1), is much less than that used by others (2–5 per cent)[3,9,11]. The specific activity, 1·13, of this purified cellulase was little higher than that of a crude commercial cellulase 0·58 ('Onozuka SS' cellulase, All Japan Biochemicals Ltd, Nishiomiya) when assayed by following the hydrolysis of carboxymethyl cellulose reductiometrically[12]. The relatively small amounts of purified cellulase capable of producing plant protoplasts may be a reflexion of its high C_1 activity[8].

Table 2. SUGAR COMPOSITION OF PLANT CELL WALLS

Suspension cultures	Glc	Gal UA	Gal	Man	Ara	Xyl	Fuc	Rha
Glycine max L.	+ +	+ + +	–	+	–	–	+	+
Rosa sp. variety 'Sceptre'	+	+ +	+ +	–	–	+	+	–
Triticum monococcum L.	+ +	+ +	+ +	+	+ +	+ +	–	–
Haplopappus gracilis (Nutz) Gray	+ +	+	+ +	–	+ +	+	+	+
Meliotus alba Desr. variety "Arctic"	+ + +	+ + +	–	+	+	–	–	–
Linum usitatissimum L.	+ + +	+	–	–	+	–	–	–

Sugar composition was determined by acid hydrolysis[5].
–, +, + +, + + +, relative intensities on paper chromatograms.

We do not know why cellulase alone or in combination with pectinases did not form protoplasts from *H. gracilis*, *Rosa* sp. and *T. monococcum*. Possibly the action of the yeast endopolygalacturonase or fungal pectate lyase could require the prior action of an arabino-furanosidase. These pectinases act respectively on low and highly esterified pectins. Though pectinases promote disaggregation of plant tissue from various species[13] and aid in cell wall degradation[9,14], they are not essential for the production of protoplasts from cells of *G. max*, *L. usitatissimum* and *M. alba*. Lipases and proteases do not seem to be essential in the formation of fungal protoplasts[12]. Presumably specific arabinose, galactose or xylose-containing hemicelluloses of the cell walls of the former species either prevented the action of cellulase against essential structural cellulose components by a physical masking action, or themselves gave sufficient structural support to the walls to prevent the formation of protoplasts. Some other factor may be necessary to initiate lysis[14].

Possible sources of other cell wall lytic enzymes (for example, hemicellulases) include (i) crude commercial enzymes and (ii) inducible enzymes produced by culturing a lytic organism in the presence of cell walls. The latter can be used to prepare specific glycosidases, active towards single plant species. We used *Streptomyces* sp. 0143[12] for the preparation of enzymes lytic towards *H. gracilis*. The fungus was grown on a chemically defined medium[12] with 0·3 per cent dry cells as a source of carbohydrate. Extracellular enzymes produced after 4 days were concentrated, desalted and tested for effectiveness in producing protoplasts, which were obtained from *H. gracilis* cells when incubated with the induced enzymes. Analyses of enzyme hydrolysates from *Haplopappus* cell walls yielded arabinose, fucose, glucose and an unidentified uronic acid. The purification of hemicellulases demonstrated to be present will be attempted when the hemicellulose components of the cell walls have been analysed structurally.

The fact that a single enzyme can be used to produce protoplasts from certain plants is encouraging in the search for a rigorously defined "protoplast-hybridization" methodology. The production of specific inducible lytic enzymes may also help in attaining this objective. We have observed regeneration of cell walls on protoplasts using commercial enzymes as others have[11,15,16], but conclusive evidence of complete cell regeneration must await the demonstration of both nuclear and cellular division.

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Biological Control of *Pistia stratiotes* L. in Western State, Nigeria

Pistia stratiotes L. is a free-floating water-weed, common on many waters in West Africa. It is capable of rapid vegetative reproduction by offshoots, and concern has been expressed over a possible infestation of the lake behind the newly closed Kainji dam on the River Niger in Nigeria where the plant is now accumulating. Our discovery of the cause of a die-back of this plant which occurs annually on enclosed and permanent waters in the Ibadan area of Western State, Nigeria, offers some hope, however, that the plant could be controlled biologically on Kainji Lake.

Systematic field observations from October 1967 to January 1969, coupled with results from experiments on the growth pattern of the plant, showed that the die-back in the Ibadan area was associated with a virus infection which reached epidemic proportions during the wet season. The sequence of events observed during this period is believed to be typical of the annual die-back.

Infected plants were rare during the dry season (November–March) and the beginning of the wet season. The infection started spreading rapidly during May, some time after the beginning of the rains, and continued until mid-July by which time no symptomless plants could be found on any of the waters of the Ibadan area. By September most of the infected plants had died, although some were still alive. During October, at the end of the rains, a few symptomless plants reappeared and were responsible for the build-up of the dry season populations.

Infected plants were readily identifiable in the field by the deep yellowing and brittle nature of their lowermost leaves and by their less vigorous growth. The infection seems to have been spread by the widely distributed aphid, *Rhopalosiphum nymphaeae* L., which occurred on *Pistia* in small numbers during the dry season but greatly increased in numbers during the wet season when the winged forms were also dispersing. Transmission of the disease by this insect has been demonstrated experimentally in the greenhouse.

The seasonal differences in the spread and effects of the disease were attributable to the population changes of the aphid coinciding with changes in climatic conditions and, perhaps more important, with changes in the nutrient status of the water. When the aphid population was enlarging and dispersing during the early rains, growing conditions for the plant were less favourable because of the cool, overcast conditions and the dilution of the nutrients in the water by the frequent heavy rains, and plants began to show signs of mineral deficiency. In these conditions spread of the infection was rapid and the weakened plants succumbed readily to the disease. During the dry season, when the aphid population was much reduced and relatively sedentary, growing conditions were better with less overcast skies and the nutrient status of the water reached its highest levels. Changes in these conditions have been shown experimentally to affect survival and growth rates of infected plants.

The symptomless winter populations are believed to have been derived from infected plants rather than seed. There is evidence that the spread of the virus to the offshoots is relatively slow and, in the better growing conditions of the dry season, the rate of vegetative propagation outstrips infection; but here we do not yet known enough.

A somewhat similar cyclical die-back of *Salvinia auriulata* Aublet, associated with a saprophytic *Alternaria* sp. and a parasitic *Spicariopsis* sp., has been described from Lake Kariba[1]. In this case circumstantial evidence suggested that the degeneration was chiefly caused by physiological–ecological factors which predisposed the plants to fungal infection.

The infection of *Pistia* does not bring complete control, only a dramatic cut-back of the population during the unfavourable conditions of the wet season. Observations suggest that the infection may be widespread in Western State, Nigeria, and, although we do not know whether it occurs elsewhere, conditions seem favourable for its existence throughout much of the forest region of West Africa. It may have caused the partial disappearance of *Pistia* from the Volta Lake in Ghana, after an initial build-up when the dam was closed; in time, it may also affect the accumulation of *Pistia* behind the newly closed Kainji dam. The climate of the Kainji area, however, is drier than that of Ibadan, and the wet season is shorter, so that the infection may consequently be less effective. If no infection appears at Kainji it would, of course, be advisable to introduce infected plants into the population to see whether some measure of control would result.

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Symbiotic Emergence of Metazoans

It has been assumed that differentiation during embryonic development is based on the regulation of gene activities. The differentiation of each cell, then, results from the activity of certain genes and the inactivity of others. Practically all theories of embryonic development rely on this assumption (see Markert[1]). It is evident that different genes are active in different cells (that is to say, haemoglobin genes in erythroblasts, thyroglobulin genes in thyroid cells, myosin genes in muscle cells). But the precise programming of cells containing thousands of genes still fails to explain the regular embryonic development, which depends not only on cellular differentiation but on precise cell divisions, cell and tissue movements, cell interactions and even cell death. Moreover, embryonic development is flexible enough to respond to injury, from the formation of complete identical twins to the fusion of two organs to form a single one.

It is perhaps necessary to explore hypotheses of embryonic development that are not restricted to the processes of cell regulation at the level of gene transcription. It is known that the instructions for embryonic development are present in the unfertilized egg. These instructions encompass all levels of biological organization, however, and, furthermore, they have changed and evolved during the evolution of metazoans. It is often assumed that metazoans evolved directly from one line of unicellular organisms. In this report, I explore the possibility that metazoans were the inevitable result of the intracellular symbiosis of many unicellular (prokaryotic and eukaryotic) organisms when they could no longer survive as single cells; metazoans were formed when the daughter cells remained together and formed intercellular symbionts. The theory[2,3] and the experimental evidence[4-6] for the occurrence of intracellular symbiosis during the evolution of eukaryotic cells have been reviewed before. Furthermore, the intracellular union of two or more cells is easily achieved in tissue cultures[7].

An intracellular symbiont could have had a better chance of surviving because it would be more adaptable to environmental or nutritional changes than any of its parent cells. It is also probable that not all the genetic information would be used during any one life cycle, but that some would be maintained and used only when required for survival. The genetic system resulting from intracellular symbiosis would provide variability for survival under changing conditions, but it also would be under pressure to lose redundant genetic information. The result could be the maintenance of integrated genetic networks, capable of switching on or off in response to external stimuli. Survival pressures would probably work towards the stabilization of such genetic networks so that their functions would be more and more mutually exclusive. The organisms would, however, remain flexible enough to utilize the different genetic networks at different times. Models of how the integrated activation of batteries of genes can produce different states of cellular differentiation have been made[8]. For survival, any one of the various states of differentiation could be switched on or off in response to the environmental requirements. For example, the amoebo-flagellate transformations in protozoans[9] may reflect the pre-existence of such intracellular symbiotic forms; at present, they can switch and use mostly the genes of one or the other of the original partners depending on the environmental requirements.

Under pressure to express simultaneously more than one of the many possible differentiated states, the descendants of one cell could have remained together, and evolved into primitive metazoans. Such a primitive metazoan would be an intercellular symbiont with the multiplicity of its genetic information expressed simultaneously, but in different cells. All the advantages of the original intracellular symbiont, that is, its capability of expressing different genetic networks at different times, were now transferred to the new organism. But now there would be an added advantage; the capability of simultaneous expression in one multicellular organism.

Then, during the further evolution of the metazoan, the successive differentiated states could be triggered by signals produced from the neighbouring cells rather than by changes in the environment. The result would be stabilization of the morphology of the symbiont. As in all symbiotic relationships, the interdependence of the partners would result in the loss of redundant functions and in increased cooperation. This was probably more apparent in the formation of the primitive tissues and organs. For example, the primitive gut was a cavity where cells cooperatively digested food by extracellular secretions. Other cells in the symbiont survived by absorbing the nutriment without a need for proteolytic enzymes. The processes of early embryonic development are similar in all metazoans. First, there is a period of rapid cell cleavage, followed by a time of cellular movement and interaction between the cells in the different regions. Tissue and cellular differentiation then begins in various regions. The hypothetical primitive intracellular symbiont as it evolved into an intercellular symbiont would have gone through these same processes.

Any hypothesis of embryonic development must explain the origin and the evolution of the developmental processes that lead to cell differentiation, besides explaining the genetic regulation which is commonly believed to account for cell differentiation. The hypothesis of intracellular–intercellular symbiosis provides an explanation of the origin of metazoans which emphasizes the survival value of the developmental processes. The origin of metazoans through intracellular and intercellular symbiosis may be illustrated by considering a simple hypothetical example (Fig. 1). For illustration, both exaggeration and simplification are used, thus diminishing the probability of such events actually occurring. Although proof of this theory must await the design of new experiments, some experimental evidence can be explained in its light.

The large amounts and the different kinds of genetic messages required for the transition from unicellular to multicellular life are the result of intracellular symbiosis, and not of mutation alone. On this basis, metazoan cells would have much larger amounts of DNA than unicellular organisms, as indeed seems to be the case[9,10]. And, because all unicellular organisms carry on much the same basic life processes, large amounts of redundant DNA would also result from intracellular symbiosis and be found in metazoans[11].

A loss of redundant DNA would also be expected, however, because the specialization of the intercellular symbiont would depend on the interdependence of its cells. We should therefore find that, in some cases, organisms considered more primitive in the evolutionary scale could have more DNA than organisms considered more advanced. For example, the lung fish contains seventeen times more DNA than the frog, and the frog has twice the amount of DNA as has the mouse[12].

If the survival of the intercellular symbiont depended on the cooperative function of neighbouring cells, then survival pressures dictated not only for the morphogenetic movements responsible for the establishment of these relationships, but also for the complete morphology attained by an organism. In this light, embryonic regulation (twinning of organs and embryos, and the organ fusion, reaggregation and formation of complete embryos from dispersed cells) may be considered an attempt of the components of the symbiont to attain the relationship that provides for maximum survival capacity.

Initially, any one cell could have given rise to a complete individual and the primitive metazoan could have reproduced by liberating a few cells. Later, however, as all the other cells in the organism specialized, so did the egg cells. They were provided not only with yolk but also "masked" messenger RNA (ref. 13) to carry out cellular proliferation

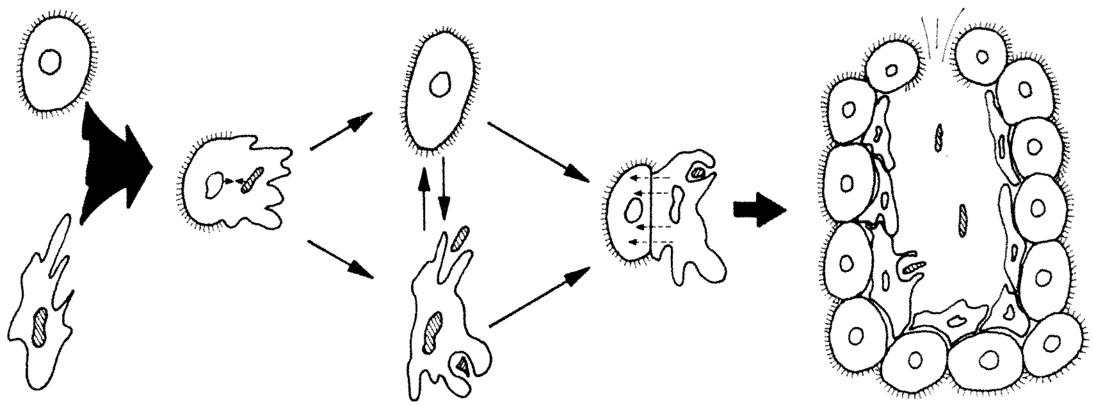

Fig. 1. If an amoeboid is considered to unite with a ciliated organism, the resultant symbiont, by being able to switch and use the genetic networks of either parent, would be at a survival advantage. For example, if the nutriment was largely particulate, the amoeboid form would be more advantageous. On the other hand, in the presence of soluble nutriments and predators, the ciliated organism would have a better chance to survive. Under survival pressures, there would be the development of mechanism for detecting changes in the environment and the capacity to switch on the appropriate genetic networks. As these mechanisms specialized, there would be the mutual exclusion of genes other than those required for a specific environment. The increased genetic information in the symbiont would also make the establishment of other morphologies possible; however, for illustrative purposes, the two extreme morphologies of the symbiotic partners are shown. As suggested in the text, the metazoan state would be established when environmental requirements made it necessary to have more than one of the differentiated cell types. In the example here, this would probably be an environment with particulate food and many predators. The formation of tissues would have resulted from further specialization and interdependence between partners.

in order to speed up the attainment of the multicellular state. Under the suggested evolutionary scheme, fertilization, which is a specialized form of intracellular symbiosis, may be considered the recapitulation of the ancestral stimulus for the emergence of metazoan life.

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Are Genetic Correlations and Environmental Correlations Correlated?

CORRELATIONS between different characters measured on individuals of a population have both genetic and environmental causes. In an extensive genetic analysis of the open-field behaviour of mice, which is a widely used index of "emotionality", we have found that genetic and corresponding environmental patterns of association among pairs of characters are similar. We suggest that this relationship may frequently occur in nature and may account for the failure of recent attempts to obtain maximally and minimally heritable factor scores by multivariate analysis.

In quantitative genetic theory[1], the observed phenotypic value of a character measured on an individual may be partitioned as

$$P_x = A_x + E_x \qquad (1)$$

where P_x is the phenotypic value, A_x is the additive genetic value and E_x is the deviation resulting from both environmental influences and non-additive gene effects. When two characters are measured on individuals from a segregating (non-isogenic) population and thus partitioned, the correlation between the observed phenotypic values may be expressed as

$$r_{Pxy} = h_x h_y r_{Axy} + e_x e_y r_{Exy} \qquad (2)$$

where r_{Pxy} is the phenotypic correlation, r_{Axy} is the genetic correlation (that is, the correlation between the additive genetic values for the two characters), r_{Exy} is the corresponding environmental (plus non-additive genetic) correlation, h is the square root of the heritability of the indicated character ($h^2 = V_A / V_P$, where V_A and V_P are the variances of the additive genetic and phenotypic values), and $e = (1 - h^2)^{1/2}$. From equation (2), when characters are measured on individuals from an isogenic population the observed phenotypic correlation is a direct estimate of r_E, because V_A and, hence, h^2 are zero. Non-additive genetic deviations within isogenic populations must also be zero; thus, when environmental correlations are estimated from isogenic populations, the resulting estimates will be based only on associations between environmental deviations.

As part of a genetic analysis of open-field behavioural characters of mice[2,3], data were obtained from 2,641 subjects including members of two inbred strains (BALB/cJ and C56Bl/6J) and their derived F_1, backcross, F_2 and F_3 generations. Individuals were tested in a brightly lit open field for three minutes on each of two consecutive days at 40 ± 5 days of age. Walls and floor of the field were constructed of white, painted 'Plexiglas'. Two sets of five light sources were beamed through holes and red filters on each of two adjacent sides of the field to photoconductive cells on the opposite side, effectively dividing the floor into thirty-six squares, 6 × 6 inches each. The number of light beams interrupted during each test was automatically recorded on counters and the number of faecal boluses deposited by each subject was counted at the conclusion of each test. The resulting daily activity scores (number of light beams interrupted) and defaecation scores (number of faecal boluses) were subjected to square root transformations. Our methods are discussed in more detail elsewhere[2].

Phenotypic correlations among the single-day behavioural scores are presented for each strain and derived generation in Table 1. These values were obtained from unweighted means of correlations calculated separately by sex and, where reciprocal crosses were involved (F_1, B_1, B_2 and F_2), by mating class. (This method of pooling was found to yield values very similar to those obtained by pooling corrected sums of squares and cross products across subclasses.) As expected, activity on days one and two (A_1A_2) and defaecation on days one and two (D_1D_2) are each positively correlated for all generations. In contrast, all correlations between single-day activity and defaecation scores are negative, again demonstrating the inverse relationship between activity and defaecation in the open field[2].

Table 1. PHENOTYPIC CORRELATIONS AMONG SINGLE-DAY OPEN-FIELD BEHAVIOURAL SCORES OF MICE FROM TWO INBRED STRAINS AND DERIVED GENERATIONS

Generation	A_1A_2	A_1D_1	A_1D_2	A_2D_1	A_2D_2	D_1D_2	N
P_1 (BALB)	0·34	−0·16	−0·12	−0·06	−0·06	0·45	64
P_2 (C57Bl)	0·20	−0·03	−0·12	−0·01	−0·08	0·17	112
F_1	0·43	−0·37	−0·18	−0·22	−0·42	0·27	415
B_1	0·66	−0·30	−0·18	−0·26	−0·36	0·13	257
B_2	0·39	−0·31	−0·26	−0·13	−0·41	0·24	315
F_2	0·60	−0·28	−0·20	−0·17	−0·44	0·36	637
F_3	0·65	−0·37	−0·31	−0·26	−0·49	0·30	841

A_1, A_2, D_1 and D_2 symbolize activity on days one and two and defaecation on days one and two, respectively.

Heritabilities of single-day activity and defaecation scores and the genetic correlations among them were estimated from parent–offspring comparisons available from data of the F_2 and F_3 generations. These estimates, as well as pooled estimates of r_P from these generations, have been published previously[3], but because the data are useful for estimating r_E and for later comparisons they are presented again in Table 2.

The primary objective of the present communication is to examine the relationship between r_A and r_E. (We are not discussing the possible association between additive genetic values and corresponding environmental deviations, that is, not r_{AE}.) From the data of the present study, r_E can be estimated in two different ways: (1) from the phenotypic correlations of the isogenic generations presented in Table 1; and (2) from substitution of the values from Table 2 into equation (2). Environmental correlations among the single-day open-field behavioural scores were estimated by both methods and are presented in Table 3. (Pooled estimates obtained from the isogenic generations were calculated by weighting the F_1 correlations by two, for the frequency of heterozygotes in the F_2 and F_3 generations is expected to be twice that of either homozygote.) Differences between corresponding estimates of r_E above and below the diagonal could be attributed to sampling error or to the fact that one set (above diagonal) comes from the correlation between environmental deviations only, whereas the other set comes from correlations between environmental plus non-additive genetic deviations. In general, however, corresponding values in Table 3 are in remarkable agreement, especially considering that entirely different methods and generations were involved in their estimation. A comparison of the values in Table 3 with those above the diagonal in Table 2 is also interesting: when the genetic and corresponding environmental correlations are compared, a similar pattern of association is observed.

Table 2. GENETIC CORRELATIONS ± $S.E.$ (ABOVE DIAGONAL), PHENOTYPIC CORRELATIONS (BELOW DIAGONAL) AND HERITABILITIES ± $S.E.$ (MAIN DIAGONAL) OF SINGLE-DAY OPEN-FIELD BEHAVIOURAL SCORES OF MICE IN F_2 AND F_3 GENERATIONS (FROM REF. 3)

	Day 1 activity	Day 2 activity	Day 1 defaecation	Day 2 defaecation
Day 1 activity	(0·28 ± 0·09)	0·94 ± 0·04	−0·51 ± 0·19	−0·89 ± 0·07
Day 2 activity	0·63	(0·09 ± 0·07)	−0·10 ± 0·41	−0·76 ± 0·24
Day 1 defaecation	−0·34	−0·23	(0·14 ± 0·06)	0·20 ± 0·41
Day 2 defaecation	−0·29	−0·47	0·32	(0·07 ± 0·06)

Table 3. ENVIRONMENTAL CORRELATIONS AMONG SINGLE-DAY OPEN-FIELD BEHAVIOURAL SCORES

	Day 1 activity	Day 2 activity	Day 1 defaecation	Day 2 defaecation
Day 1 activity	—	0·35	−0·23	−0·15
Day 2 activity	0·59	—	−0·13	−0·25
Day 1 defaecation	−0·30	−0·13	—	0·29
Day 2 defaecation	−0·21	−0·44	0·34	—

Estimates above diagonal were calculated from pooled phenotypic correlations within isogenic generations, whereas those below diagonal were obtained from solution of equation (2).

Although our data are relatively extensive, the number of possible comparisons between r_A and r_E is not large. Nevertheless, we wish to suggest that r_A and r_E may often be correlated and that such a correlation might, with hindsight, be predicted from evolutionary considerations. Consider, for example, the first pair of characters listed[1] in Falconer's Table 19.1: the genetic correlation between milk yield and butterfat yield is 0·85 and the environmental correlation is 0·96. The magnitude of the genetic correlation indicates that cattle with high additive genetic values for milk yield will also have high additive genetic values for butterfat yield; that is, genic substitutions which result in higher milk production also lead to higher butterfat production. In a similar manner the large environmental correlation indicates that an environment conducive to high milk yield will also promote high butterfat yield. From the standpoint of biological efficiency, it would seem most reasonable that correlated characters should respond similarly to both genetic effects and environmental deviations. Although several of the cases listed by Falconer might serve as counter examples to this argument, the standard errors of estimates of genetic correlations are notoriously large. Nevertheless, individual exceptions may occasionally occur.

Further evidence for the relationship between r_A and r_E is provided by the results of attempts to obtain maximally and minimally heritable factor scores by multivariate analysis of genetic and environmental matrices. Such attempts have been relatively unsuccessful, at least in terms of the degree of separation achieved. Thompson[4,5] has discussed the importance of attempting to isolate factors which maximally represent either genetic or environmental sources of variation. Multivariate analysis of genetic and environmental correlation matrices seems to provide a promising approach which Loehlin and Vandenberg[6] have used in their twin study of human cognitive ability. But factors extracted from two matrices, one representing mostly genetic effects and the other environmental, were basically similar. In a multivariate analysis of matrices of genetic and environmental correlations among morphological and production characters of swine[7], resulting components describing the genetic and environmental correlational patterns were also similar. Thus, in separate studies which used widely different characters and species, multivariate analyses of genetic and environmental correlation matrices achieved little separation. Such lack of separation would be expected if there were an element-by-element association between the two matrices, that is, if r_A and r_E were correlated.

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Dislocation of Tones in a Musical Sequence: a Memory Illusion

Computer-generated tonal sequences have several advantages for investigations of immediate memory: the stimulus parameters are simple and can be exactly controlled, and the items cannot be readily rehearsed. Previous studies of memory for pitch have investigated the effect on the differential threshold of blank intervals between the standard and comparison tones[1-3] and of one interpolated tone[4,5].

In the recognition of simple musical sequences the order of the component tones is usually supposed to be well retained; and this assumption is especially important to musical analyses based on information theory[6]. I have, however, found that there can be a rapid loss of such order information in a very simple tonal sequence; namely, the traditional musical scale.

The procedure was as follows. A subject listened to a test tone, which was followed by a sequence of four intervening tones and, after a pause, by a probe tone. The subject was instructed to try to remember the test tone, ignore the four intervening tones if he wished, and then to judge whether the probe tone was or was not of the same pitch as the test tone. All tones lasted for 200 ms. The first five tones were separated by intervals of 300 ms, and there was a 2 s pause before the probe tone (see Fig. 1). The tones were all equally loud and taken from an equal-tempered scale ($A = 435$) ranging just over an octave, from middle C to the C♯ an octave above. When the test and probe tones differed in pitch, the difference was always a semitone (higher in half the instances, and lower in the other half): this is well above the recognition threshold in the absence of intervening tones, even with much longer delays between test and probe[1-3]. The intervening tones were chosen randomly except that no sequence contained repeated tones unless they were specified by the experimental conditions. All the test and probe tone pitches were equally represented in all conditions.

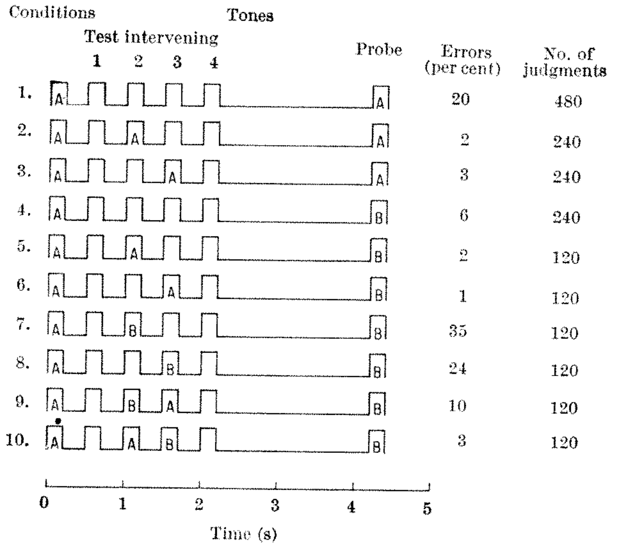

Fig. 1. Arrangement of tonal sequences in the different experimental conditions, with number of judgments and percentage of errors made in each.

Tones were generated by a Wavetek oscillator controlled by a PDP9 computer, and were recorded on tape. Subjects were selected on the basis of obtaining a score of at least 80 per cent correct on a short tape containing similar sequences. The data from the two days of the experiment were averaged.

The experiment investigated the effect on recognition memory of inserting, in the intervening sequence, tones of the same pitch as the test and probe tones. The different experimental conditions are shown in Fig. 1. Here, *A* represents the pitch of the test tone, and of the probe tone when the two are identical. *B* represents the pitch of the probe tone when it differs from *A*. The results (Fig. 1) show that insertion in the intervening sequence of a tone at the same pitch as the probe tone has a striking effect. When the test and probe tones differ in pitch, and neither of these pitches is included in the intervening sequence, errors are only 6 per cent (condition 4). Errors increase to 24 per cent with insertion in the third serial position of the intervening sequence of a tone at the same pitch as the probe tone (condition 8); and to 35 per cent with insertion in the second serial position (condition 7). This effect is significant on a sign test at the 0·01 level. Further, when the test and probe tones are identical in pitch, insertion in the intervening sequence of a tone at that pitch causes a dramatic reduction in errors (2 per cent and 3 per cent in conditions 2 and 3 respectively, compared with 20 per cent in condition 1). This is also significant on a sign test at the 0·01 level.

I propose that subjects showed an increased tendency to err in conditions 7 and 8 because, although they correctly recognized that the probe tone pitch had occurred, they were unable to localize it correctly in the intervening sequence. The same loss of order information could also account for the increased tendency to judge the test and probe tones as the same in conditions 2 and 3 (although the latter finding could result from a trace strengthening effect of repeating the test tone); and this consideration might also be applied to the decreased number of errors when both the test and probe tone pitches are included in the intervening sequence (conditions 9 and 10) rather than the probe tone pitch alone (conditions 7 and 8).

It might be thought that the subjects did not initially hear the sequences correctly; but in a control experiment the identical sequences were played, with the probe tone 2 s before the test tone, and the subjects had no difficulty in making correct judgments.

The conclusion that memory for tonal order deteriorates rapidly, even in the traditional musical scale, can be reconciled with the unquestionable importance of order in tune recognition by assuming that we process tonal information on the basis of higher-order attributes[7] and rapidly discard absolute pitch information.

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Book Reviews

SAFETY FIRST

Perils of the Peaceful Atom

The Myth of Safe Nuclear Power Plants. By Richard Curtis and Elizabeth Hogan. Pp. xiv + 274. (Gollancz: London, March 1970.) 52*s*.

In the *Perils of the Peaceful Atom*, Richard Curtis extends his range of satire, fiction and non-fiction and, with co-author Elizabeth Hogan, sets out to show that atomic energy presents to man new and horrific dangers, and that government agencies and others in the United States have developed atomic energy unnecessarily, too fast and at too great a cost. Many of the issues raised are particular to the United States, but the general claim of horrific danger, if true, would demand the urgent attention of all nations.

The case unfolds in the following way. First, and the main theme, a pen picture of the unknown and terrible dangers of radioactivity. This is followed by a number of sections describing briefly some accidents, problems of waste storage and transport, a reference to insurance, the heavy demand on cheap uranium supplies, much about the frailty of man and a special disparagement of US administration. The story concludes with the authors' solution to the world power demand of the future, as magnetohydrodynamics, magnetoplasmadynamics, electrogasdynamics, fuel cells, hydroelectric plants, tidal power, geothermal energy and solar energy. "As for clouds, a number of means are being developed for storage of collected solar energy, so that reserves can be built up, literally, for a rainy day."

Because the case rests on many quotations or statements attributed to others, it is important to examine their validity. It is said of the accident at Windscale in 1957 that "authorities had to seize all milk and growing foodstuffs in a four-hundred-square-mile area". In fact, milk was controlled but there was no seizure of any foodstuffs or limitation of its use. This is followed by, "According to Sir John Cockcroft . . . considerably more radioactivity was released at Windscale than is released during an explosion of a Hiroshima-type atom bomb". This statement was not made by Sir John Cockcroft but appeared in 1958 as an erroneous deduction by a science correspondent. The radioactivity released, as compared with the bomb, was less than 1 per cent in terms of iodine and less than 10^{-4} of total gamma activity (measured at one day).

The authors describe the accident to the FERMI reactor in 1966 as an "event as close to Armageddon as this country (USA) has ever known". The International Commission on Radiological Protection has published its evaluation of risks from radiation. Using these and other data, it can be shown that the release of all radioactive material contained in the reactor on that occasion, if carried in the direction of Detroit, a city of 1·5 million, might have led to a risk of about 3×10^{-5} per person that some effect from radiation might appear within twenty years. This is not Armageddon. A similar use of a warlike comparison appears later in the suggestion that a nuclear accident may "wreak death and harm on a level potentially surpassing Hiroshima and Nagasaki". This comparison is not valid. The radiation harm was the result of the direct and intense radiation from the weapon; this does not occur in a reactor accident.

The nuclear industry recognizes the long-term problem involved in the storage of concentrated fission products. There are means for rendering these wastes into insoluble agglomerates of a glass-like material which would make them reasonably secure even against violent upheavals of nature. It may be questioned whether enough effort is invested in solving future problems, but the authors' case is not improved by using language intentionally distorting facts such as, "at present the solutions are stored in tanks whose seams groan under strains metal was never meant to bear".

The authors' treatment of the effluent problems of the industry is confusing. They quote an increase in radioactivity of plankton, fish and ducks as being a thousand to a million times greater. But greater than what ? Also that humans in the Columbia area may have more than 4,000 picocuries of zinc-65; this is only a thousandth part of the permitted body burden and is only one thirtieth of the radioactive potassium carried by all men.

The many inaccuracies and distortions detract from the value of the book. This is a pity because the subject is important and deserves more rigorous selection and checking of the material used. The exaggerated charges made could set back any move towards an open discussion of safety problems. The authors claim that "many AEC pamphlets present over-simplified pictures, almost word cartoons that insult the adult mentality". If so, perhaps these pictures and the attempt to show by over-simplification that the maximum credible accident will cause no harm may be techniques developed, perhaps unwisely, by an industry fearing that any recognition of risk could lead to exaggerated distortions as in the present book.

Although the evidence presented is often erroneous or highly distorted, a rebuttal of the charges made in the book does not imply that there is no need for constant vigilance in determining and applying adequate safety criteria at all stages of an atomic energy programme. A more general form of the authors' argument appears in their foreword: "We are convinced that those who favor our present commercial nuclear power program do not recognize how far we are in actual practice from the high standards and ideal conditions which even the most enthusiastic proponents acknowledge to be essential to public health and safety". Without sharing their conviction expressed in such extreme terms, I accept that no technologically based industry can afford to be complacent about safety. The book may serve a purpose in helping to underline, if rather hysterically, the high standards of engineering, operation and technical assessment required.

F. R. Farmer

COMMUNICATION AND INFORMATION

Information, Mechanism and Meaning

By Donald M. MacKay. Pp. iv + 196. (The MIT Press: Cambridge, Massachusetts, and London, February 1970.) 65*s*.

From time to time, but too seldom, a scientist looks up from his bench or his desk and talks to the educated layman in non-technical terms. Professor MacKay's book records a number of such informal exchanges with the intelligent public. It is a collection of papers, articles and broadcasts, bound together by an introduction and marginal notes, on communication and information. In a book of this kind there is bound to be a certain amount of repetition and even of inconsistency, but this adds verisimilitude to an intellectual autobiography.

To me, the questions raised by the author are often more interesting than his answers. One such question arises from the fact that orthodox information theory, as expounded by Shannon, has no place for the concept of meaning. MacKay devotes quite a lot of space to examining the concept of information, and does some discreet advertising on behalf of his own distinctions between metrical, selective, semantic and structural information content. Unfortunately these distinctions are usually ignored by writers on information theory, but this may be because the idea of information as "negative entropy" lends itself to mathematical development and technical application where the other concepts do not. Perhaps time will redress the balance.

In discussing the nature of meaning the author expresses himself in terms appropriate to automata with well defined "transition probability matrices". Much of what he has to say is clear and cogent, but one may perhaps doubt whether this particular mathematical concept is robust enough to carry such heavy philosophical burdens. Nevertheless, MacKay raises conceptual questions which cannot be ignored, and his book will undoubtedly interest everybody who is seriously concerned with the relation between minds, brains and automata.

CHRISTOPHER LONGUET-HIGGINS

D'ARCY THOMPSON RIDES AGAIN

Symmetry and Function of Biological Systems at the Macromolecular Level

Edited by Arne Engström and Bror Strandberg. (Proceedings of the Eleventh Nobel Symposium held August 26–29, 1968, at Södergarn, Lidingö, in the County of Stockholm.) Pp. 436. (Interscience (Wiley): New York and London; Almqvist and Wiksell: Stockholm, October 1969.) 280*s*.

SYMMETRY, it seems, is in. Possibly it is the delayed impact of the work of the past ten or so years on the structures of complex self-assembly systems, such as viruses and flagella, and the symmetry principles which govern them, that has caused the space groups of late to trip so lightly from biochemists' tongues. Symmetry, it has to be said, is a slender thread to link so diverse a set of articles as are contained in this book, however, and frequently its relevance is lost to view. As with all such volumes, one imagines that the true begetters are the publishers rather than the organizers. Published a year after the event, the papers fall into three categories—those that have in the meantime been published in reputable journals (and a few that are still about to be), the well-worn lecture review (and one or two in this volume present a remarkably threadbare appearance), and the ruminations of the patrician mind, which might otherwise be denied to the hungry scientific proletariat. At best, this last kind can justify the publication of symposium volumes. At worst, and more often, one retains only the impression that all the elaborate and expensive machinery of international congresses and symposia is capable of generating nothing more useful than the occasional blinding cloud of steam. I confess that I addressed myself to the Eleventh Nobel Symposium without enthusiasm. In the event I found much more than one could have hoped that was new, stimulating and even entertaining.

The substance of the book falls into six sections. The first, "Basic Views on Symmetry", is chiefly remarkable for an unaccountable two-page colour plate of the heads of "Australians and Ocean People", but includes also a short, concentrated and stylish exposition by a mathematician (H. S. M. Coxetter) of "The Symmetry Properties of Helices and Concho-spirals". The second section is about "Prediction of the Conformation of Macromolecules", and contains the articles by Scheraga, Ramachandran and Liquori, without which no symposium is nowadays complete. There follows "Interaction between Subunits in Polymeric Proteins", which is an indigestible concoction of X-ray crystallography, electron microscopy and physical chemistry. The paper by Schuster and Ilgenfritz on the kinetics of haemoglobin oxygenation is one of the most substantial and interesting in the book, however, and is a good example of the impact of the Göttingen school on current thinking in molecular biology. "Symmetry and Cooperativity in Biological Membranes" shows the extent to which these have now been sucked into the vortex of allosterism. Nevertheless, however dyspeptic one's response to this prospect, and however much adrenaline, for that matter, one may release on encountering a quotation from Molière at the head (though everyone, of course, to his taste), it must be said that the paper by Changeux is an elegant piece of work. A nicely wrought intellectual structure, balanced on a very pin's tip of experimental fact, it is persuasive to a remarkable degree, and whether or not the concepts turn out in the end to be valid, I do not doubt that it will have its effect on thinking in the field.

The remaining two sections of the book are on "Assembly and Structure of Viruses", which contains particularly an impressive article by Klug, and "Aspects of Macromolecular Assignments in Muscle", with a magisterial chapter by Casper and Cohen, on the nature and significance of polymorphism in protein aggregates, as the centrepiece. There is also a short appendix by Klug called "Point Groups and the Design of Aggregates", which sets out in a dozen pages the principles of symmetry and the manner in which they apply to viruses as the archetypal subunit systems.

There are, then, five or six articles in this volume which are not to be had elsewhere and which encapsulate some of the best achievements in molecular biology of the past few years. There are a good many others which are, I suppose, none the worse for having been published more than once, and only one that struck me as altogether bad and misleading. On this reckoning the book scores a good deal higher than most symposium volumes. It comes at a point in time at which some fundamental ideas incubated over many years by crystallographers, protein chemists and others have come to maturity. The finesse of modern techniques aside, it is D'Arcy Thompson and the great anatomists of the nineteenth century writ small. There are many things here which molecular biologists and their students will wish to ponder on.

W. B. GRATZER

GENETICS BY COMPUTER

Computer Applications by Genetics

Edited by Newton E. Morton. (Proceedings of a Conference sponsored by the University of Hawaii, and the Genetics Study Section, National Institute of General Medical Sciences.) Pp. ix + 167. (University of Hawaii Press: Honolulu, Hawaii, October 1969.) $10.

THIS collection of papers is dedicated to Dr L. H. Snyder and is concerned with the applications of computers in genetics with a strong bias towards human population genetics. The sixteen papers cover a wide variety of topics, and the authors are acknowledged experts in each field. Some of the papers contain a description of the relevant computer programs. The discussion following the reading of some of the papers is included.

There are two papers on Monte Carlo simulation. One, by Levin, discusses the use of simulation in studying the effects of sampling, selection, migration and competition on gene frequencies. Levin discusses in an appendix to his paper some approximate short-cut simulation methods without stressing how much these may reduce the value of the stochastic accuracy that is one of the great advantages of exact simulation. The other paper, by Latter, is an excellent, although perhaps over optimistic, discussion of the use of selection experiments and simulation studies to discriminate between different models of quantitative genetic variation.

There are seven papers on the statistical and computational problems involved in analysing pedigrees, in segregation analysis, and in estimating breeding values, gene frequencies and inbreeding coefficients.

Other more abstract but thorough papers by Morton and Cotterman are concerned with genetic changes in populations isolated by distance and with complex relationships between genotypes and phenotypes. A paper by A. W. F. Edwards gives a rather discouraging review of the state of genetic taxonomy. This will come as no surprise to the more traditionally inclined.

There are interesting papers by Renwick on the use of Bayesian-type methods in studying genetic linkage in man and by W. R. Bodmer and others on the association analysis of a leucocyte system. Renwick emphasizes the need for a study of the non-randomness of genes in linkage maps.

The volume concludes with papers on the simulation of deterministic evolutionary changes in three-locus genetic systems and of equilibrium conditions in certain trimorphic self-incompatible systems.

This book raises certain questions about the place of model-building and simulation studies in biology. An attempt to build a mathematical model of a biological system can often suggest deficiencies in knowledge about that system. This can, and should, lead to further observation or experimentation rather than simulation. There are dangers in attempting to simulate a system when there is too little knowledge about that system. This means that the models that are constructed are, inevitably, too flexible and have far too many unknown parameters in them. These models will, by their very nature, fit the data although they may be low in explanatory or data-summarizing power. My own feeling is that simulation and model-building should only be used when a fair amount is known about the biology of the situation and a model can be constructed that is well founded, fairly specific and contains a small enough number of parameters that they can be satisfactorily estimated by whatever data are available. I do have doubts whether some of the systems studied in this set of papers have reached a stage where model-building and simulation can profitably be applied. Latter's paper contains a useful discussion of some of these points.

This volume will clearly be of interest to all those concerned with genetics and the use of computers in genetics. It will also prove a stimulating experience to those who, although unfamiliar with genetics, are interested in the present state of the subject. There are many interesting questions asked, and by no means have all of them been answered. R. N. CURNOW

INTEGRATED GENETICS

Genetic Organization

Edited by Ernest W. Caspari and Arnold W. Ravin. (A Comprehensive Treatise, Vol. 1.) Pp. xi + 525. (Academic Press: New York and London, December 1969.) 271*s*.

THIS is the first volume of a proposed multi-volume treatise which will deal with the current state of knowledge in genetics. The reason given for producing it (when there are already so many good texts on advances in genetics) is that the discipline seems to be fragmenting, like most biological subjects of any age; so that molecular, developmental and evolutionary geneticists are losing touch with each other's work. Accordingly, the purpose of the treatise is to provide a medium whereby students of genetics can integrate recent advances in all fields into a single coherent structure, and also to reveal gaps in understanding which may indicate profitable fields of research.

In the first section, L. C. Dunn covers the history of genetics in terms of the main streams which have contributed to our present knowledge. The prose is turgid, but cannot conceal the authority of this most experienced genetic historian. Next follows a section by Henry M. Sobell on the structure of the nucleic acids; this is excellently clear and up to date. In section three, J. H. Taylor gives a masterly exposition of a field which he has made very much his own, namely the structure and duplication of chromosomes. In section four, Walter F. Bodmer and Andrew J. Darlington discuss linkage and recombination at the molecular level. This is a lucid account of recent work on microorganisms which sheds light on these topics and on the present state of knowledge on the enzymology of DNA breakage and repair.

In the fifth section, Sterling Emerson writes on linkage and recombination at the chromosome level. This is a very technical account of evidence from a wide range of organisms. This subject is never easy reading, and comprehension is not helped by the complexity of the data which are presented. The review is perhaps written from a more personal viewpoint than others in this volume, and includes a large addendum containing the author's views on the acquisition and handling of certain genetic data. In the sixth and last section, Rhoda F. Grell reviews knowledge of meiotic and somatic pairing. Here, as in the previous section, the density of the genetic jargon makes reading hard work but cannot conceal either the author's deep knowledge of the field or the exhaustiveness with which this knowledge is deployed.

My feeling about this book is that it is essentially a collection of up to date and authoritative reviews at a high intellectual level. While the clarity of exposition varies considerably between the different authors, the thoroughness with which they have performed their task is unquestionable; the relevant literature has been thoroughly dredged (no doubt all the latest techniques of information retrieval have been used). Whether the book will effect its avowed objects is uncertain. Few scientists will have a broad enough background to read it all with understanding (and even fewer will be able to afford to buy it, certainly in Britain). But many will find this volume (and the ensuing ones if the high standard is maintained) invaluable as a reference book, particularly in their own and related fields. It is to be hoped, therefore, that many libraries will be willing to find the money to purchase it. E. H. R. FORD

MEDICAL DIAGNOSIS

Physical Principles of Ultrasonic Diagnosis

By P. N. T. Wells. (Medical Physics Series, Vol. 1.) Pp. viii + 282. (Academic Press: New York and London, November 1969.) $12.50; 80*s*.

IN the course of some ten years ultrasonic diagnosis has advanced from a rather tentative method in the hands of a few pioneers to an accepted technique complementing, but not replacing, the use of X-rays. This progress has involved close collaboration between clinicians, physicists and engineers. The physics is highly specialized but it must be sufficiently understood by all three groups if the potential of the method is to be exploited. A book designed for this purpose is therefore timely and welcome.

It must be said at once that the difficulty of the task has not been shirked by pretending that the subject is simple. To set it out in full detail would require a very large volume, and the author has compromised by summarizing the essentials in a number of well defined sections which are supplemented by a bibliography of more than four hundred selected literature references. Starting from simple wave motion, he deals in succession with the passage of ultrasonic waves through matter, with transducers and with the ultrasonic field. He then discusses the various techniques which are available including the very important ones based on pulse echo and on the Doppler effect. It is at this stage that he introduces a number of typical clinical applications to show the scope as well as the limitations of the methods. Finally, he discusses the possible biological hazard in the use of ultrasonics.

On the whole, these topics are covered adequately and in a balanced manner. A little more information about the physical processes involved in biological damage would have been welcome, but this is a part of the field which is perhaps expanding too rapidly for simple summary. Possible future developments have not been overlooked, for example, the use of ultrasonics in holography of the human body. This book should do much to encourage further development in this important branch of medical diagnosis.

C. B. ALLSOPP

BLADDER CANCERS

Occupational and Environmental Cancers of the Urinary System

By W. C. Hueper. Pp. xix + 465. (Yale University Press: New Haven and London, November 1969.) 180*s*; $20.

DR HUEPER has in effect written two books within the cover of one. The first book is an account of the factors that are known or are suspected to be involved in the development of bladder tumours. The general belief in clinical circles is that bladder tumours are partially, if not completely, produced as the result of chemical constituents in the urine which act on the urinary epithelium and produce neoplasia. This observation, which was first made in 1895 by Rehn, has been confirmed on numerous occasions both experimentally and in human beings. Up until 1937 it was generally believed that the chemical responsible was aniline because many of these tumours occurred in factories where aniline was manufactured, but the work of Hueper in 1937 established that an intermediate product in the production of aniline, β-naphthylamine, was in fact one of the most potent carcinogens available. Not merely was it the first carcinogenic agent that could be identified chemically but it was also a carcinogenic agent producing bladder tumours when applied to experimental animals and to man.

Hueper presents, in this book, a concise and detailed statement about the experimental work of β-naphthylamine and many other similar chemicals which are also carcinogenic. The references are extensive and very comprehensive. There is hardly an experimental paper written on the subject of bladder neoplasia that is not mentioned and discussed in detail.

The aetiological factors, such as smoking, disordered tryptophan metabolites, geographical distribution, social class, and the like, are covered in detail in a critical analysis which evaluates the evidence for each factor and poses many problems to both the clinician and the epidemiologist.

The second book has been written between the lines. It is the story of a man who has spent his life fighting for the recognition that chemicals were dangerous, that industries using these chemicals were producing bladder cancer, and the story relates the frustrations, the difficulties and the woeful obstructions that seem to have been put in his way in order to prevent him establishing the truth of the dangerous nature of these chemicals. This struggle did not occur in one country alone. When Hueper describes the investigation in France carried out by Desoille he states "the reason for the development of bladder tumours became evident when Desoille attempted to investigate . . . as it became apparent that bladder tumours were, in fact, present in the factory. Desoille was asked by the management to leave the premises". In Italy, the manufacturers were among the last of the large-scale producers of β-naphthylamine to abandon making this highly potent carcinogen (1961). The Swiss industry ceased manufacture in 1938, the Germans in 1942, the English in 1952, the Russians in 1950, and most of the factories in the United States in the decade between 1950 and 1960. β-Naphthylamine is still being manufactured in Japan and benzidine is manufactured in Russia and Japan. On p. 80 Hueper states "these data were not obtained without some resistance from the industries concerned. The delay in the publication of the results . . . of the epidemiological investigations . . . which were on hand in 1949, but which were not placed on record until 1954".

This story of the difficulties which have been encountered by research workers in elucidating the true facts about industrial aetiology is one that should be much more widely known among scientists. In Britain, the manufacture of β-naphthylamine stopped in 1949 (not 1952 as stated in the book) and the manufacture of α-naphthylamine and benzidine in 1968–69. It would seem, from reading this book, that Britain has established a leading role in the control of these carcinogenic agents, legislation to prevent manufacture and importation, and compensation for workmen who, as a result of exposure to these chemicals before the recognition of the dangers, have developed bladder cancer.

D. M. WALLACE

CALCIUM PHYSIOLOGY

Mineral Metabolism

An Advanced Treatise. Edited by C. L. Comar and Felix Bronner. Vol. 3: Calcium Physiology. Pp. xvi + 548. (Academic Press: New York and London, October 1969.) 229*s*.

THIS work does not fit easily into any of the categories into which scientific books can usually be classified. It is neither a textbook, a monograph, a work of reference nor the proceedings of a symposium. It is essentially a collection of seven brilliant essays on various aspects of calcium physiology which combine a reasonable survey of previous work with the forceful presentation of a particular thesis. Careful selection of authors and strong editing yield a high degree of internal consistency with a marked emphasis on mathematical treatment.

Marshall's opening chapter on measurements and models of skeletal metabolism sets the tone for the whole work and constitutes a rigorous analysis of bone turnover by a physicist with a strong biological orientation. None the less, it is noticeable that when biological and physical concepts conflict, he shows a marked bias towards the former and almost seems to imply that bone turnover, as understood by bone physiologists, is not a meaningful concept. In fact, he uses technical operations to define biological concepts, instead of the general procedure of defining the concepts first and considering the way in which experimental techniques can measure them. It follows from this approach that some of his terminology, such as augmentation rate, may find little acceptance in the biological field.

Subsequent chapters are perhaps less controversial but equally stimulating. Richelle and Onkelinx review the composition and structure of bone mineral competently and impartially. They lay bare the essence of the controversy as to whether bone mineral is a calcium deficient apatite or a mixture of apatite and octacalcium phosphate, and discuss the available evidence regarding the nature of the carbonate component. They deal with thermodynamic aspects of the relation between bone mineral and tissue fluids and discuss the role of pyrophosphate and other inhibitors, concluding that the concept of a solubility product constant is inadequate to explain the observed data. The authors' known work on the frequency distribution of specific gravity and mineral content of bone powder is restated and discussed in relation to X-ray diffraction patterns and infrared spectra.

Winegrad's review of calcium and striated muscle is a useful, brief survey of intracellular calcium. Walser's chapter on the renal excretion of alkaline earths brings together some useful published data, the discussion of which is rather brief, but which is supported by a bibliography extending to thirty pages. Wasserman's and Taylor's contribution on calcium absorption, on the other hand, is less complete as a review of the subject, but provides an admirable account of their work on the calcium-binding protein.

The concluding chapters are by Manery on calcium and membrane and by Copp on calcium homeostasis. Manery's chapter overlaps to some extent with Winegrad's but may be read as complementary to it. Copp's contribution is inevitably a restatement of his previous views with only minor additions from recent work, but, as would be expected, it is readable and well informed.

The work as a whole is impressive, powerful and somewhat controversial, but it is stimulating and on the whole highly readable. B. E. C. NORDIN

HOST-PARASITE RELATIONS

Immunity to Parasitic Animals

Edited by G. J. Jackson, Robert Herman and Ira Singer. Vol. 1. Pp. ix + 292. (North-Holland: Amsterdam; Appleton-Century-Crofts (Educational): New York, 1969.) 128*s*.

THE first book on the immunology of parasitic infections was that written by Taliaferro more than forty years ago. Since that time there have been notable advances in our knowledge of host-parasite relations, but in spite of all the new information the overall pattern remains obscure and is full of apparent anomalies and unanswered questions. In particular, attempts to control parasitic disease by immunological means have largely proved unrewarding. There are a few vaccines against protozoa and helminths which find application in veterinary practice, but none has proved sufficiently safe and potent to be used in human medicine. There are many reasons for this comparative failure. Not the least is the fascinating intricacy of the life cycles of many of these organisms, providing, at the same time, delight and frustration for those working in this field.

The editors of this review have planned a two volume work, attempting a wide coverage of the subject. The first volume is largely devoted to the immunity (resistance ?) shown by plants and by invertebrate and cold-blooded vertebrate hosts to infection with animal parasites. The various contributors make it plain that these are neglected fields of research. Thus—"Our knowledge of the immune reactions of poikilothermic vertebrates to parasitic protozoa is little more than scant", and "No published reports were found on immunity of amphibians and reptiles to metazoan parasites". What reports there are in these fields tend to be widely dispersed (a paper on agglutinating antibodies in the trout appeared in a journal entitled *The Progressive Fish Culturist*!), and the contributors are to be congratulated on achieving some synthesis of the literature. Their reviews will help to fill awkward gaps in the teaching on host-parasite relations and, it is hoped, will encourage more workers to explore these fields.

Standing apart from the general body of the volume is the review by J. F. A. Sprent on evolutionary aspects of immunity in zooparasitic infections. Sprent attempts to define for the immunologist the special nature of the parasitic association and, for the parasitologist, what is meant by immunity. I doubt whether he succeeds, but the result is a discussion which will greatly interest those already familiar with the two disciplines.

Most workers in the field of parasite immunity are concerned with immunity shown by mammalian and avian hosts. While welcoming this first volume, they will look forward with more interest to the publication of the second. R. J. TERRY

BLOOD PARASITES OF SLOTHS

The Haemoflagellates of Sloths

By J. J. Shaw. (London School of Hygiene and Tropical Medicine, Memoir No. 13.) Pp. xii + 132 + 13 plates. (H. K. Lewis: London, 1969.) 70*s*.

THE sloth is a curious, exotic animal, whose name indicates its habits. It has an equally strange parasite—*Endotrypanum schaudinni*—a trypanosome which inhabits the red blood corpuscles of the animal. This unusual monograph gives an account of the sloth and the parasite. Actually, two genera of sloths are concerned—the two-toed genus *Choloepus* with two species, *C. hoffmanni* and *C. didactylus*, and the three-toed *Bradypus* with two species, *B. griseus* and *B. infuscatus*. Moreover, the researches of the author have demonstrated that two species of *Endotrypanum* (*E. schaudinni* and *E. monterogei*) and two species of trypanosomes (*T. preguici* and *T. leeuwenhoeki*) exist in the blood of these animals. The complex is analysed in relation to the biogeographical background: the habits of the sloths, the type of forest where infection takes place, and the biting arthropods which transmit the haemoflagellates.

The result of this approach is the elucidation, at least in part, of an interesting tropical problem. The author's own work was carried out in the forests of Costa Rica, Panama and Colombia; in addition, past records from Central America, Venezuela, French Guiana and northern Brazil are considered. *Endotrypanum schaudinni* was found in a third of more than a hundred specimens of the two-toed sloth; a new species, *E. monterogei*, was described from two specimens of the sloth; two new trypanosomes were also described from the animal. The Noguchi-Adler test was applied to determine the relationships of these organisms to each other and to a local human strain of *Leishmania* and of *T. cruzi*. They all seem to be immunologically distinct, though *Endotrypanum* is nearer to *Leishmania* than to the three trypanosomes. This affinity is confirmed by the results of cultivation at different temperatures *in vitro*, and by the apparent role of sandflies (*Lutzomyia* spp.) in transmission. The fine structure of *E. schaudinni* is described and the truly intra-erythrocytic position of the organism was confirmed; a peculiar pigment, allied perhaps to malarial haemazoin, was found in the parasites.

The haemoflagellates of sloths have puzzled observers since the discovery of the first in 1908. This monograph is a major contribution to the subject, and although of a specialist nature should prove of interest to the landscape epidemiologist and general biologist.

The book is profusely illustrated by a coloured frontispiece and thirteen line drawings of the organisms, by various photographs of the sloths and their habitats, and by photomicrographs. P. C. C. GARNHAM

READ, LEARN AND INDIGEST

A Textbook of Practical Biology

By George Usher. Pp. 208. (Constable: London, January 1970.) 45*s*.

THIS textbook is a programme of directions for basic practical work designed for A-level candidates particularly where overcrowded classes make it impossible for the student to have adequate individual attention. The author modestly proffers it as a compendium of work he has successfully carried out, the fruits of 12 years' experience in teaching A-level biological sciences.

The arrangement is systematic and covers the essentials of most A-level syllabuses in biology, botany and zoology, together with a great deal of simpler work usually regarded as pre-O-level. Although it is claimed that the student should be able to carry out the work on his own, some of it, notably that on *Drosophila* and on the examination of capillary blood flow and of muscle response in the frog, would need a good deal of supervision and technical support. The sequence is logical. It begins with the physical properties of colloids, the chemistry of cells, enzymes, cell structures and cell division. Then, after some simple experimental work on respiration, the student is taken through a routine dissection of the rat, being first reminded, lest an examination question on it comes as a shock, not to skimp a thorough review of the external features. Gross structure of particular organs of the body is well related to microscopic detail in this section and it is followed by a chapter in which practical work in physiology and histology is correlated. Taxic response, growth and the action of growth hormones are illustrated here using protozoa, arthropods and frog tadpoles. The student is next led through a series of representative types which show the variety of invertebrate and vertebrate animals, then on to the morphology and anatomy of flowering plants and to standard experiments in plant physiology. Variety in the plant kingdom is shown by a further sequence of traditional types. A short section on soil constituents, physical properties and larger soil organisms ends in brief suggestions for simple comparative field work which might be a sound introduction to basic ecological concepts. Meiosis is studied from prepared slides and squashes and there are interesting suggestions for graphing numbers and measurements to illustrate variation. Work on *Drosophila* is cursory and the same might be said of frog and chick embryology.

A useful appendix gives recipes for reagents and media required in the course, and there are lists of apparatus, chemicals and biological materials mentioned in the text.

This is no "open-ended enquiry" approach, although, by following it, students should be helped to see things for themselves and have their expectations confirmed. They are essentially put through a rigorous, systematic routine to fulfil examination requirements and the author makes no bones about it. This course, faithfully followed, supplemented by good text and background reading and backed by adequate field work, would doubtless be crowned by success.

The deliberately simple, occasionally crude, line diagrams are claimed to be of the type and standard expected from a candidate in an examination. Some are eminently satisfactory, but a wealth of excellent examples in recent publications leads us to demand very high quality in this field. Incidentally, I prefer unbroken to dotted lines for joining labels and the parts they indicate. Students are offered very sound advice, especially in observing and recording rat anatomy (page 24), on the desirability for measurement and minimal shading. Directions in the main are very complete and explicit, but there are occasional lapses: "make a 0·5 per cent solution of starch by boiling in water" (page 10). There are some printing errors.

E. J. VINNICOMBE

COLOURFUL GAME BIRDS

A Guide to the Pheasants of the World

By Philip Wayre. Pp. 176. (Hamlyn: London and New York, February 1970. Published for Country Life Books.) 63*s*.

THIS is a thoroughly practical book and carries the authority of an ornithologist and aviculturist who has made a close study of his subject over many years. While not supplanting the larger work by Delacour (who here contributes a foreword) or the earlier monograph by Beebe, it gives an up to date summary of the main facts about each of the forty-eight species constituting the Phasianini tribe of the Phasianidae, and some overall remarks about each of the sixteen genera in which they are placed. There are six introductory chapters of a more general kind, dealing chiefly with avicultural aspects, and three appendices.

The tribe includes not only all the birds that are called pheasants, but also the tragopans, junglefowl and peacocks, with some others that have special names. Apart from the results of introductions by man, they are almost restricted to Asia, chiefly from the western Himalaya eastwards to Japan and Borneo; the natural range of one species extends westwards just into Europe and another species, not discovered until 1936, is exceptional in being an inhabitant of equatorial Africa. The habitats vary from tropical rain forest to mountains 15,000 feet above sea level, and from reed beds to steppes, but most of the species are forest dwellers.

The birds are of substantial size and good to eat; the males are in most instances highly colourful; and the specific ranges are often very restricted. These facts have helped to bring at least sixteen of the species within danger of extinction at the present time. Fortunately, they mostly thrive in captivity and many of them readily breed. The Pheasant Trust, of which Philip Wayre is honorary director, has been notably successful in building up captive stocks; and in 1937 it was able to return thirty Swinhoe's pheasants to Formosa, where the species is endemic and was on the verge of extinction in the wild state. Such efforts, of course, need to be reinforced by local measures to conserve suitable habitats and to protect the birds.

There are good colour illustrations of the males of forty-six species by J. C. Harrison. No females are figured, but their plumages, generally cryptic, are described in the text. The volume is well produced.

LANDSBOROUGH THOMSON

CRYSTALS AND ROCKS

Introduction to Mineralogy

Crystallography and Petrology. By Carl W. Correns. Second edition, in cooperation with Josef Zemann (Part 1) and Sigmund Koritnig (Mineral Tables). Translated by William D. Johns. Pp. xi + 484. (Allen and Unwin: London; Springer–Verlag: Berlin, January 1970.) 105*s*.

IT is exceptional nowadays for a single textbook to provide adequate treatment, even at an elementary level, of all the diverse fields of knowledge required by the modern mineralogy student. But this Professor Correns achieved with considerable success in the first edition of his book which appeared in 1949. The subjects covered were crystallography, crystal chemistry, optics, petrology, weathering and soil formation, ore deposits and geochemistry. Then, as now, the extensive mineralogical tables were compiled by Professor Koritnig and mineralogy was approached through petrology. In the second edition an extensive revision of part one, "Crystallography", has been undertaken by Professor Zemann; that of part two, "Petrology", has remained with Professor Correns. The new volume is about a fifth larger than its predecessor, but extensive deletions of older matter have permitted substantial amounts of new material to be added. Additional topics include volatiles, metamorphic facies, and isotope geochemistry. The layout of the tables has been improved and they now include a considerable amount of new information not mentioned in the text.

Part one is divided into four sections, crystal symmetry (misnamed mathematics); crystal chemistry; crystal physics (chiefly optics and X-ray crystallography); and crystal growth and dissolution. Symmetry is treated formally by way of morphology, but later, ideas of lattice symmetry are developed which serve to introduce space groups. The crystal chemistry is just what is required, but the X-ray section would have been strengthened by a fuller treatment of intensities, accurate determination of cell dimensions, choice of radiation, and some mention of diffractometers. In a section on crystal physics, surely an attempt should be made to justify the indicatrix. Part two begins with an interesting account of crystallization processes and phase diagrams up to ternaries; it then leads on to hydrous systems. The sections on igneous and metamorphic petrology are much too condensed, but the account on the role of volatiles is quite the best to appear in an elementary text. There are further chapters on sedimentary rocks, metasomatism, the development of tectonites, and geochemistry.

The account seems to have lost little in translation, the diagrams are excellent and the paper, although still of two types, is of good quality. A bibliography is provided as well as author and subject indexes. The work throughout is written with authority and reaches a high standard, but one is left with the feeling that this vast field would have been covered much better by two books.

I. D. Muir

OPTICS FOR UNDERGRADUATES

University Optics

Vol. 1. By D. W. Tenquist, R. M. Whittle and J. Yarwood. Pp. viii + 349. (Iliffe: London, October 1969.) 70*s*.

In the preface the authors describe this text as "intended to cover the requirements in the subject of optics for the student preparing for Part 1 of an honours degree in physics, a general degree in science or ancillary physics to an honours degree in chemistry or other main discipline". Of the material covered in volume one, the first three chapters deal with geometrical optics and optical instruments. These chapters deal with these difficult subjects clearly and concisely. Refractometry and photometry take up nearly one-third of the volume and it is this extensive treatment of practical optics which distinguishes this book from the many other available texts. The velocity of light is given a chapter to itself, which seems a little excessive. The wave theory of light is followed by sections on interference, diffraction and resolving power.

There are worked examples throughout the text and questions from various university degree examinations are included at the end of each chapter, with answers to the numerical problems at the end of the book. The treatment throughout is traditional, in contrast with other recent undergraduate books in optics. While there is nothing particularly novel in either presentation or material, the writing is thorough and accurate. Special attention is paid to practical details, particularly in those sections dealing with optical measurements and instruments. There are plenty of clear, well-drawn diagrams which supplement the text, but it is a pity that the publisher did not choose a better quality of paper.

To sum up, this is a useful book which, while suitable for applied physics and ancillary physics courses, probably does not present sufficient challenge for a first year undergraduate intending to specialize in physics.

D. J. Bradley

Obituaries

Mrs M. J. Richards

Mrs M. J. Richards (Dr Maud Norris) will be remembered chiefly for her work as a locust entomologist, in which she had a world-wide reputation. It was Mrs Richards who discovered the maturation pheromone of the desert locust in 1954. Her association with locusts and the Anti-Locust Research Centre came about almost by accident and in spite of the fact that her early work had been on pests of stored products. When the Anti-Locust Research Centre became an independent research institute in 1945, the director at that time, Dr B. P. (now Sir Boris) Uvarov, was looking for a scientist to run the laboratory. Mrs Richards expressed interest, was given a research grant in 1946 and from then until December 1969 never gave up locust work.

Maud Richards was born in Plymouth in 1907, the daughter of a naval officer, and was educated at several schools including Cheltenham Ladies' College. At King's College, London, she obtained first class honours in zoology in 1928. She became a demonstrator at King's College and then held a DSIR grant in the Department of Zoology and Applied Entomology of Imperial College, where she obtained her PhD degree. It was also here that she met her husband, Professor O. W. Richards, who was then a lecturer in the department, and she was married in 1931. From 1932–34 she was a research assistant at Imperial College and took up what was to remain the major theme of all her work, the reproductive processes and associated behaviour of insects. Her three papers on the stored products moth *Ephestia*, published in 1932, 1933 and 1934 and still quoted today, typify her broad approach and include studies of the structure and functions of the reproductive organs, the effect of food and external factors, and physiology.

After 1934, when she stopped working full time, she did part time work in the British Museum (Natural History) on the *Zoological Record* as well as voluntary work at the Pest Infestation Laboratory in Slough. In 1937 she accompanied her husband to British Guiana, sharing his Leverhulme grant, to collaborate on the biology of social wasps; a notable joint paper resulted later. In 1946 Mrs Richards joined the ALRC as a research fellow, was then appointed an SSO and finally promoted to PSO, which she remained until her death this year. While there she published more than twenty-five important papers on locust biology and physiology, and built up an international reputation for her work on the effects of grouping and phases in locust biology. She made important observations on the effects of density, food and photoperiod on locust reproduction and maturation, but clearly something else more elusive was involved and in 1954 her patience and persistence were rewarded with the discovery of a chemical influence, the maturation pheromone. This was a finding of great importance and was one of the first demonstrations that this type of compound existed.

Mrs Richards's research stimulated further work in the ALRC and in many other laboratories in this field, resulting directly in the discovery of more pheromones. She herself found others connected with reproduction and, recently, a chemical functioning in gregarious oviposition behaviour of locusts. Indirectly her work generated interest in chemicals biologically active in locust reproduction, particularly plant chemicals that trigger maturation.

She was now well known internationally, and was awarded the DSc of the University of London in 1964. She was much in demand for international symposia, treating this recognition with characteristic modesty; she was also interested in field work overseas, enjoying these visits not only for the experience itself but because they provided her with insights for further laboratory work. During the 1960s she went abroad frequently. In probably the most interesting and arduous of her trips she and Professor Richards joined the Royal Society and Royal Geographical Society Expedition to the Mato Grosso in Brazil in 1968. She passed off the hardships of the life there with characteristic calmness and said that she was much more interested in the great variety of grasshoppers that she found in the forest than in the difficulties of camp life.

Mrs Richards was one of the longest-serving members of the Anti-Locust Research Centre; she had a quiet, friendly, unassuming manner and never sought the limelight. But she could be roused when scientists, be they colleagues or not, drew unwarranted conclusions from insufficient data, and then she gently but firmly drew attention to the lapse. Her help and counsel were much in demand by her colleagues at home and overseas, and she was always ready to assist them. She will be sadly missed not only in the centre but by locust scientists all over the world.

Correspondence

A-levels and University Performance

SIR,—I would like to comment on D. G. Bagg's interesting article on A-levels and university performance (*Nature*, **225**, 1105; 1970), and to take a different view of the predictive value of examinations based upon a less literal interpretation of the results of analysis and consequently reaching more conventional conclusions.

I have made an investigation of the predictive validity of examinations from university entry qualifications to final degree for all candidates for the honours degree in mechanical engineering of the University of Salford from 1959 to 1964. This study is continuing; there is, however, a time delay of five years to allow for students who require five years to complete the normal four-year course. As in Bagg's work, the study is longitudinal, but attempts to predict only from one sessional examination to the following sessional or final examination taken one year later. The predictive criterion used is the average mark per script obtained by each candidate in each of the successive examinations. This criterion was selected because it gave significantly better correlation from year to year than the mark in any individual subject and also because the frequency distribution of average mark per script was found to be very close to a normal distribution for sessional and final examinations, thus satisfying an important condition for a linear regression model.

Table 1

Entry qualification	No. of candidates	Average mark per script	Correlation coefficient
GCE Advanced level in three or more subjects, including maths and physics	243	56·7	0·286
GCE Advanced level in maths and physics only	187	53·1	0·292
Ordinary National Diploma	40	58·0	0·461
Ordinary National Certificate (internally examined)	155	55·3	0·285
Ordinary National Certificate (externally examined, with four subjects)	121	54·8	0·422
Ordinary National Certificate (externally examined, with three subjects)	65	53·1	0·213

The condition of normal frequency distribution was not satisfied by the marks obtained in the entry qualifying examinations, these marks tending to be biased towards the minimum acceptable standards for the course. For this reason and because of the variety of entry qualifications offered by students, which in turn led to relatively few students in some of the categories of entry group, linear regression equations were not calculated to predict first-year sessional examination performance from entry qualification performance. However, product-moment correlation coefficients between entry qualification performance (divided into six categories of qualification) and average mark per script obtained in the first-year sessional examination were calculated and provide an indication of the degree of relationship between these variables. The average mark per script obtained in the first-year sessional examinations for all candidates in each of the six entry qualification categories was also calculated and is shown, with the correlation coefficients, in Table 1.

The regression equations and correlation coefficients for performance in sessional and final examinations of candidates in successive years of the course are shown in Table 2, in which $x_{1,2,3,4}$ denotes the average mark per script obtained by a candidate in the first, second and third year sessional examinations and the final examinations respectively.

Table 2

Sessional examination	No. of candidates	Correlation coefficient	Predicted mark
First to second	265	0·660	$x_2 = 11{\cdot}82 + 0{\cdot}745\ x_1$
Second to third	232	0·723	$x_3 = 6{\cdot}58 + 0{\cdot}916\ x_2$
Third to final	233	0·736	$x_4 = 4{\cdot}63 + 0{\cdot}887\ x_3$

The correlation coefficients shown in Tables 1 and 2 indicate that performances in successive university examinations are more closely related than performance in GCE Advanced level or other entry qualifying examinations with subsequent performance in university examinations. It would be surprising if this were not so, in view of the relative homogeneity of the university situation compared with the variety of examining boards, schools and social backgrounds of the candidates before university entrance.

This factor, in conjunction with the reasonable surmise that undergraduates are, by the time they begin university courses, already highly selected from the general population as far as examination performance is concerned, leads me to conclude that final degree performance cannot be expected to bear marked relationship to performance in A-level examinations as a whole. This relationship can be expected to weaken with performance in a single A-level subject and to become negligible if factors affecting performance which are common to two or more subjects are removed in the regression analysis. This effect is clear in the multiple regression equations presented in Bagg's analysis. This is not to argue that A-level grades are unreliable and possibly hazardous predictors of future academic performance but that to expect more than a general indication of academic ability is to expect too much. That A-level performance does provide such an indication has been demonstrated by Petch[1], who states that of a sample of 3,523 students who entered a university in October 1956, after being examined by the Joint Matriculation Board, nine out of ten justified their selection by subsequently completing degree courses and that incidence of premature termination of courses was higher for less well qualified groups of students than for groups which obtained better results at Advanced level. An additional inference which may be drawn from Table 1 is that A-level performance is not the only indicator of the potential ability of undergraduates, and I suspect that this conclusion is as valid for other disciplines as it is for engineering.

Yours faithfully,

R. R. PLATT

Department of Mechanical Engineering,
University of Salford.

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[1] Petch, J. A., *GCE and Degree, Part I. Joint Matriculation Board* (Manchester, April 1961).

University News

Geoffrey V. Ball, head of the Department of Ophthalmic Optics, has been appointed professor of ophthalmic optics in the **University of Aston in Birmingham**, and **Dr Michael R. W. Brown**, Bath University of Technology, has been appointed professor and head of the Department of Pharmacy, also in the **University of Aston in Birmingham.**

ERRATUM. In the review of "A Short History of Botany in the United States" (*Nature*, **225**, 1078; 1970), the second and third sentences of the first paragraph should read: "The eleventh International Botanical Congress was held in Seattle in August 1968. This volume outlines the growth of knowledge . . ."

ERRATUM. In the article "Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from Pulsars" by Hong-Yee Chiu and Vittorio Canuto (*Nature*, **225**, 1230; 1970), equation 5 should read:

$$\alpha_\omega = \frac{8\pi^3 c^2}{n_r{}^2\omega^2}\, 2m \int_0^\infty \left[f(p_z{}')\, \frac{\partial}{\partial p_z{}'} \left(\frac{Q_\omega(p_z{}')}{p_z{}' + p_z}\right) + f(-p_z{}')\, \frac{\partial}{\partial p_z{}'} \left(\frac{Q_\omega(p_z{}')}{p_z{}' - p_z}\right)\right] \mathrm{d}p_z{}' \qquad (5)$$

ERRATUM. In the article "The Man who Lived with Australopithecines" (*Nature*, **225**, 1188; 1970), it was implied that the stone industry recovered from the Swartkrans cave site was contemporaneous with the Oldowan culture from Bed I at Olduvai, thus making it about 1·75 million years old. The comparison should, however, have been with the much younger culture from Bed II at Olduvai.

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Exchange: Furnished 3 or 4-bedroomed house/apartment near the University of California, San Francisco, required for one year from July 1, 1970. Would exchange for a furnished 4-bedroomed house with central heating in Highgate, London N6, very near Highgate tube station. Please contact Dr M. de Swiet, Medical Unit, University College Hospital Medical School, University Street, London WC1 (telephone 01-387 5861).

Vacant: From July 1 to September 30, 1970. Furnished house, 3 bedrooms, central heating, 5 miles from St Andrews and the University. Please contact Dr David Scott, Department of Zoology, The University, St Andrews, Fife, Scotland.

Wanted: From early June to either end of July or preferably end of September, a 3-bedroomed house for botanist, wife and two children returning to Cambridge for study leave. Within three-mile radius of city centre preferred. Please contact W. Hadfield, Tea Research Association, Jorhat-8, Assam, India.

Wanted: Furnished flat or house in London with easy access to Imperial College, from beginning of June 1970 to end of January 1971, for American professor and family. Possibility exchange accommodation London/Harvard. Please contact Mrs B. Davidson, C.C.A., Imperial College, London SW7 (telephone 01-589 5111, ext. 1408).

Wanted: Small house or flat for married couple in the vicinity of the MRC Clinical Research Centre at Northwick Park, Harrow, Middlesex, for any period of time exceeding 3 months from September 15, 1970. Please contact Dr Philip Davies, Department of Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA.

British Diary

Monday, April 20

Education (6 p.m.) Sir James Tait, Royal Society of Arts, at John Adam Street, Adelphi, London WC2. (First of three Cantor Lectures on Training for Industry.)

Large Scale Integration in Microelectronics (7.30 p.m.) Mr D. D. Jones, Institution of Electronic and Radio Engineers, at the BBC Engineering Training Centre, Woodnorton, Evesham.

PAM Graded Concentric Windings for 2-speed Single-phase Induction Motors (5.30 p.m.) Dr A. R. W. Broadway and Mr M. R. Weatherley, Institution of Electrical Engineers, at Savoy Place, London WC2.

Perfumery (three-day symposium) British Society of Perfumers; and the Society of Cosmetic Chemists of Great Britain, at the Grand Hotel, Eastbourne, Sussex.

Recent Developments in Extrusion (6.45 p.m.) Mr D. Grant, Plastics Institute, at the Waldorf Hotel, Cooper Street, Manchester.

Skynet (10 a.m. colloquium) Institution of Electronic and Radio Engineers, Joint IEE/IERE Communications Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Tuesday, April 21

Automation of Gas Chromatography (8 p.m.) Dr D. R. Deans, Society for Analytical Chemistry; and the Royal Institute of Chemistry, at Constantine College of Technology, Borough Road, Middlesbrough.

Cresol—Its Production and Use (6.30 p.m.) Dr D. McNeil, Society of Chemical Industry, at the University, Southampton.

Lasers and Their Applications (7.15 p.m.) Institution of Electrical Engineers, at Harwood House College, Bletchley, Bucks.

Organic Photochemistry (two-day symposium) University of Manchester Institute of Science and Technology, Sackville Street, Manchester 1.

Pyro-electric Materials for Infra-red Detectors (5.30 p.m.) Dr E. H. Putley, Institution of Electrical Engineers, at Savoy Place, London WC2.

Solar Studies with special reference to Space Observations (two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Visual Topology (5.30 p.m.) Mr Edward Ironmonger, Royal Institution at 21 Albemarle Street, London W1. (Lecture for Sixth Form Pupils from Schools in London and the Home Counties. To be repeated on April 22, 28 and 29.)

Why Hybrid Computers? (10 a.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

Wednesday, April 22

Annual Meeting (6 p.m.) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Automatic Analysis—an Industrial Viewpoint (6.45 p.m.) Mr P. G. Jeffery, Society for Analytical Chemistry; and the Royal Institute of Chemistry, at Luton College of Technology, Luton.

Logic as the Science of Mathematical Reasoning (5.30 p.m.) Professor Abraham Robinson (Yale University), University College London, in the Engineering Theatre, Malet Place, London WC1. (Further lectures on April 27 and May 1.)

Schools Project Technology (7.30 p.m.) Institution of Electronic and Radio Engineers, in the Department of Electrical Engineering, The University, Liverpool.

Real-Time Computer Model (Business Games) Mr D. Simpson, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Science and the Rule of Thumb Approach (7.30 p.m.) Mr G. H. Hutchinson, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Telecommunications Support for the Apollo Program (5.30 p.m.) Mr Lorne M. Robinson, Institution of Electrical Engineers, at Savoy Place, London WC2.

Thursday, April 23

Automatic Trains on the Victoria Line (7 p.m.) Mr R. I. M. Arthurton, Institution of Electronic and Radio Engineers, at Medway College of Technology, Chatham, Kent.

Carbon Fibres (7.30 p.m.) Mr N. Judd, Plastics Institute, at the Old Nags Head, Jackson Row, Lloyd Street, Manchester, 2.

CIMAC—Survey of Noise (6 p.m. discussion) Institution of Mechanical Engineers, Combustion Engines Group, at 1 Birdcage Walk, London SW1.

Comparison of Chromatographic Techniques (2.30 p.m.) Society for Analytical Chemistry, Chromatography and Electrophoresis Group, at the Portsmouth Polytechnic, Burnaby Road, Portsmouth.

Quasars and Radiogalaxies (5.30 p.m.) Professor F. Hoyle, FRS, Institution of Electrical Engineers, at Savoy Place, London WC2 (61st Kelvin Lecture.)

The Photoelectron Spectra of Some Molecules Containing the C≡N Group, Mr R. F. Lake and Sir Harold Thompson, CBE, FRS, and

Vibrational Relaxation in Pure CO_2 and CO_2 Mixtures, Mr C. J. S. M. Simpson and Mr T. R. D. Chandler (4.30 p.m.) Royal Society, at 6 Carlton House Terrace, London SW1.

Friday, April 24

Complexolytic Photoreactivity and Low-Energy Photosensitization (1 p.m.) Professor G. O. Schenck, Royal Institution, at 21 Albemarle Street, London W1.

Experimental Techniques for the Detection of Ion Movement in Solids, Society for Electrochemistry, Solid State Sub-Group, at the Atomic Energy Research Establishment, Harwell, Didcot, Berks.

Low-light Vision (2.30 p.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

Models and Molecules (9 p.m.) Professor W. D. Ollis, Royal Institution, at 21 Albemarle Street, London W1.

Non-standard Analysis. I. Analytic Theory of Polynomicals (5.30 p.m.) Professor Abraham Robinson (Yale University), University College London, in the Engineering Theatre, Malet Place, London WC1.

Spring Scientific Meeting (11.30 a.m.) Fisheries Society of the British Isles, in the Large Meeting Room of the Zoological Society, Regent's Park, London NW1.

Monday, April 27

Apprenticeship and Industrial Training (6 p.m.) Mr Cyril R. English, Royal Society of Arts, at John Adam Street, London WC2. (Second of three Cantor Lectures on Training for Industry.)

Contact Problems in Machines using Thyristor Assisted Communication (5.30 p.m.) Professor J. J. Bates, Mr S. Stanway and Mr R. F. Sansum, Institution of Electrical Engineers, at Savoy Place, London WC2.

Escalators and Travolators (6 p.m.) Mr C. W. Hardie, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Gunn Domain Logic (5.30 p.m.) Dr H. L. Hartnagel, Institution of Electrical Engineers, at Savoy Place, London WC2.

Quasi-stellar Objects (5.30 p.m.) Professor G. Burbidge (University of California), University College London, in the Physics Theatre, Gower Street, London WC1.

The Evolution of a Microbial Metabolic Pathway (5.30 p.m.) Professor Roger Y. Stanier (University of California), University College London, in the Botany Theatre, Gower Street, London WC1.

Reports and Publications

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Water Resources Board. TN 9 (revised): Metrication. 61. T.N. 11: The Evaluation of Errors at Flow Measurement Stations. By R. W. Herschy. Pp. 31. (Reading: Water Resources Board, 1969.) [22

BBC Engineering Monograph No. 80: An Automatic Method for the Measurement of Reverberation Time. By Dr M. E. B. Moffat, and N. F. Spring. Pp. 18. (London: BBC, 1969.) 5*s*. [22

Accelerating Innovation. (Papers given at a Symposium held at the University of Nottingham, March 1969, arranged by Aslib in collaboration with the Ministry of Technology and the Research Associations.) Pp. 64. (London: Aslib, 1970.) 35*s* net (30*s* net to Aslib members). [22

Science Education in the Region—1970: a Guide to Courses in Universities, Polytechnics and Technical Colleges. Pp. 43. (London: London and Home Counties Regional Advisory Council for Technological Education, 1970.) 8*s*. [32

Bulletin of the British Museum (Natural History). Entomology. Vol. 24, No. 5: A Revision of the Genus *Catoptropteryx* Karsch (Orthoptera: Tettigoniidae). By J. Huxley. Pp. 127–170 + 1 plate. (London: British Museum (Natural History), 1970.) 30*s*. [42

British Steel Corporation. BISRA Annual Report for the year ending September 30th, 1969. Pp. 87. (London: The British Iron and Steel Research Association, 1970.) [42

Science Research Council. List of Research Grants Current on October 1, 1969. Pp. 123. (London: Science Research Council, 1970.) [52

Youth Exchanges: The Way Ahead. By Alan Evans. Prepared by Political and Economic Planning for the European Educational Research Trust. Pp. 63. (A Europe House Publication.) (London: Political and Economic Planning, 1970.) 20*s*. [52

Marconi-Elliott Microelectronics. MSI and Memory Elements Product Guide. Pp. 16. (Witham, Essex: Marconi-Elliott Microelectronics Ltd., 1970.) [52

Genesis Two ("Naught but Zeus")—The Semele Theory of Planetary Origin. By Douglas Rookes. Pp. 40. (London: Ardilla Books, 1970.) 10*s*. [62

Imperial College of Science and Technology (University of London). Sixty-second Annual Report of the Governing Body, 1968/1969. Pp. vi + 186. (London: Imperial College of Science and Technology, 1970.) [92

University of Leeds and the United Leeds Hospitals. An Experiment in Hospital Catering Using the Cook/Freeze System. Pp. 40. (Leeds: Procter Department of Food and Leather Science, The University, 1970.) [92

Building Research Station. Current Paper 51/69: Accuracy of Measurement with Steel Tapes. By R. M. Milner. Pp. 4. (Reprinted from *Building*, 1969, Sept. 19.) (Garston, Watford: Building Research Station, 1969.) *Gratis*. [92

Ministry of Transport: Road Research Laboratory. RRL Report LR 282: Toughened Glass Windscreen Injuries in Car Occupants. By E. Grattan, N. G. Clegg and J. A. Hobbs. Pp. 6 + 6 plates. (Crowthorne, Berkshire: Road Research Laboratory, 1969.) *Gratis*. [92

Other Countries

Science Council of Canada. Special Study No. 8: Scientific and Technical Information in Canada, Part 2. Chapter 2: Industry. Pp. x + 80. (Ottawa: Queen's Printer, 1969.) $1.25. [141

India: Council of Scientific and Industrial Research. Annual Report of the Central Mining Research Station, 1968/1969. Pp. 250. (Dhanbad, Bihar: Central Mining Research Station, 1969.) Rs. 5. [141

Arthur D. Little, Inc., Decisions. Pp. 24. Directions. Pp. 24. (Cambridge, Mass.: Arthur D. Little, Inc., 1969.) [141

India. Forest Research Institute, Dehra Dun. Indian Forest Leaflet No. 182 (Timber Engineering): Test Results and Recommendations on Strength of Nail-Joined Timber Roof Trusses for Residential Buildings. Part 1: 3 to 6 Metres, Destructive Tests. By N. J. Masani and R. K. Gupta. Pp. 14. (Delhi: Manager of Publications, 1969.) Rs. 3.40; 8*s*; $1.23. [151

Norsk Polarinstitutt. Skrifter Nr. 148: The Turbulent Transfer Over an Antarctic Ice Shelf. By Torgny E. Vinje. Pp. 55. (Oslo: Norsk Polarinstitutt, 1969.) [161

Trace Mineral Studies with Isotopes in Domestic Animals. (Proceedings of a Panel on the Use of Isotopes in Studies of Mineral Metabolism and Disease in Domestic Animals organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, held in Vienna, 28–31 October 1968. (Panel Proceedings Series.) Pp. 151. (Vienna: International Atomic Energy Agency; London: HM Stationery Office, 1969.) 104 schillings; 33*s* 4*d*; $4. [161

Advisory Council on College Chemistry, Stanford University. Catalogue of Instructional Films (16 mm, Super 8, and 8 mm) for College Chemistry. (Serial Publication No. 42.) Pp. 196. (Stanford, Calif.: Advisory Council on College Chemistry, The University, 1969.) [161

International Association of Universities. 1969 World List—Universities; other Institutions of Higher Education; University Organizations. Pp. xviii + 496. (Paris: International Association of Universities, 1969.) [191

US Department of the Interior: Geological Survey. Water-Supply Paper 1850-B: Floods of June 1965 in South Platte River Basin, Colorado. Pp. iv + 64 + plates 1–4. (Washington, DC: Government Printing Office, 1969.) $1. [191

Berichte des Deutschen Wetterdienstes. Nr. 114 (Band 24): Beitrag zum Problem der Beziehungen zwischen Wettergeschehen und Blutgerinnung. Von Paul R. Bock. Pp. 37. Nr. 115 (Band 15): Langjährige Monats- und Jahresmittel der Lufttempratur und des Niederschlags in der Bundesrepublik Deutschland für die Periode 1931–1960. Von Hans Schirmer. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1969.) [191

World Health Organization. Technical Report Series, No. 435: Biological Components of Human Reproduction: Studies of their Variations in Population Groups—Report of a WHO Scientific Group. Pp. 41. (Geneva: World Health Organization; London: HM Stationery Office, 1969.) 3 Sw. francs; 6*s*; $1. [201

Harvard University Program on Technology and Society. Fifth Annual Report 1968/1969. Pp. 82. (Cambridge, Mass.: Harvard University, 1969.) [201

La Coopération Internationale: Culturelle, Scientifique et Technique—Bulletin d'Informations et de Documentation, Octobre 1969. Pp. 217. Abonnement pour les Numéros 1 et 2 (Année 1970). (Nice: Faculté de Droit et des Sciences Economiques de l'Université de Nice, 1969.) 50 francs. [201

Unesco. Annual Summary of Information on Natural Disasters, No. 1, 1966. Pp. 81. (Paris: Unesco, 1970.) 14 francs; 21*s*; $3.50. [201

Science, Technology, and Public Policy: a Selected and Annotated Bibliography. Edited by Lynton K. Caldwell. Assisted by William B. DeVille and Gertrude W. Lindesmith. Vol. 2: Articles in Journals. Pp. xii + 544. (Bloomington, Indiana: The Program of Advanced Studies in Science, Technology, and Society, Department of Government, Indiana University, 1969. Prepared for the National Science Foundation.) [201

US Department of Commerce—Environmental Science Services Administration. Atlantic–Pacific Oceanographic Laboratories—Collected Reprints—1968. 39 reprints. (Washington, DC: Government Printing Office, 1969.) $3.25. [211

Publications de l'Institut Suisse de Météorologie. Nr. 13: Contribution à la Méthodologie Granulométrique des Aérosols Amicoscopiques. Par A. Junod. Pp. 70. Nr. 14: Untersuchungen zur Quantitativen Bestimmung von Niederschlagsmengen Mittels Radar. Von J. Joss, Karin Schram, J. C. Thams und A. Waldvogel. Pp. 37. (Locano-Monti: Centrale Meteorologica Svizzera, 1969.) [211

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UNIVERSITY OF QUEENSLAND
AUSTRALIA
CHAIR IN CIVIL ENGINEERING

Applications are invited for appointment to a Chair in Civil Engineering. This position has become vacant following the death of Professor J. H. Lavery. There are two Chairs of Civil Engineering in the University, and Professor G. R. McKay is Head of the Department.

The Professor's special interest should be in Structures, and he will be responsible for all departmental activities in this area, including undergraduate and postgraduate teaching and research. He will also be required to participate in the administration of the Department as a whole. In addition, he will be expected to maintain an effective liaison with the local profession, and to assist in the administration and development of existing testing and professional advisory services. He may be required to advise the University on building matters.

He should have broad professional experience within the specified area, and this should include competence in design, analysis, construction and testing procedures.

The salary for a Professor is $A12,000 per annum. (Salary scales are at present under review.)

The University provides superannuation similar to F.S.S.U., housing assistance, study leave and travel grants for a permanent appointee of Lecturer status and above.

Additional information and application forms will be supplied upon request to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, WC1H OPF.

Applications close in London and Brisbane on May 22, 1970. (1600)

SOUTH WORCESTERSHIRE HOSPITAL MANAGEMENT COMMITTEE
SENIOR TECHNICIAN II

required for new expanding biochemistry department at Worcester Royal Infirmary (Ronkswood Branch). Modern apparatus including isotope equipment available. Technician taking F.I.M.L.T. or Special examination in biochemistry might be considered with prospect of promotion to Senior Grade II on gaining qualification.

Applications stating age, qualifications and experience, naming two referees to Hospital Secretary, Worcester Royal Infirmary (Castle Street Branch) Worcester. (1578)

BIRKBECK COLLEGE
(UNIVERSITY OF LONDON)

Applications are invited for the post of LECTURER IN ZOOLOGY. Candidates who have training and research interests in physiology will be preferred. Salary scale: £1,240 by £115 to £2,045 by £115 to £2,850 per annum, plus London allowance of £60 per annum; the initial salary will not exceed £1,470 per annum.

Further particulars may be obtained from the Secretary, Birkbeck College, Malet Street, W.C.1. Closing date: May 4, 1970. (1547)

APPLICATIONS ARE INVITED FOR ADmission to graduate programmes leading to Ph.D. or M.Sc. degrees. Teaching assistantships and fellowships are available to students with B.Sc. degrees and to persons with professional degrees. Research emphasis is on the biochemistry of calcified tissues, with particular emphasis on the protein matrix of these tissues.—For further information write to the Chairman, Committee on the Graduate Studies, Faculty of Dentistry, The University of Alberta, Edmonton 7, Alberta, Canada. (1549)

CSIRO

RESEARCH ENGINEER /SCIENTIST (AERODYNAMIC INVESTIGATIONS)

AUSTRALIA

DIVISION OF MECHANICAL ENGINEERING

GENERAL The Division of Mechanical Engineering of the Commonwealth Scientific and Industrial Research Organization, located at Highett, Victoria, has extended its activities to include aerodynamic research, with an emphasis on fan and duct flows. Experimental facilities, including air moving equipment and instrumentation, have recently been commissioned and are currently being used for research on axial flow fans, short high efficiency diffusers, aerodynamic noise, and a variety of airflow problems associated with mine ventilation, cooling towers, air conditioning, etc. As part of the expansion programme, initial steps have been taken in the design of a moderately large return-circuit wind tunnel.

DUTIES The appointee will be required to participate in the current diffuser research programme which has as its main aim the development of high efficiency short diffusers. The investigations include basic experimental studies and specific tasks undertaken for industry in respect to application aspects.

QUALIFICATIONS Applicants should have a Ph.D. degree or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability. Previous experience in boundary layer studies is desirable but not essential.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The appointment will be initially for a fixed term of three years, after which, if mutually desired, either an extension of tenure or appointment for an indefinite period will be considered. The appointment will be conditional upon passing a medical examination and an initial probationary period of twelve months may be specified. An indefinite appointment carries with it Commonwealth Superannuation Fund or Provident Account privileges. Further details supplied on application.

Applications, (quoting Appointment No: 430/283), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64-78 Kingsway, LONDON, WC2B 6BD by the *22nd May, 1970.*

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(1553)

WESTFIELD COLLEGE
(UNIVERSITY OF LONDON)

DEPARTMENT OF ZOOLOGY

Applications are invited from men and women graduates for the post of LECTURER IN ZOOLOGY from October 1, 1970.

Further particulars should be obtained from the Deputy College Secretary, Westfield College, Kidderpore Avenue, Hampstead, N.W.3, to whom applications (six copies), stating date of birth, qualifications and experience, together with the names of two referees, should be submitted by May 22, 1970. (1522)

IMPERIAL COLLEGE

Applications are invited from qualified chemists, physicists, chemical engineers or mineral technologists for the position of research assistant to work on the practical development of a new flocculation process. The project is sponsored by English China Clays Ltd. It will require a critical analysis of the theory of flocculation and the design and evaluation of new plant and offers an unusual challenge for an inventive scientist. Salary £1,500 to £2,000 per annum plus F.S.S.U.

Applications to Professor M. G. Fleming, Department of Mineral Technology, South Kensington, London, S.W.7, within one month of the date of this advertisement.

(X1353)

For further Classified advertisements see page vi

Research opportunities in the Netherlands with Philips

PHILIPS

The PHILIPS RESEARCH Laboratories,
near Eindhoven are looking for

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mathematicians, mathematical physicists, applied mathematicians, mathematical engineers and numerical analysts, to work on mathematical problems arising in physics, chemistry, mechanical engineering, electrical engineering, thermodynamics, hydrodynamics, combinatorial and logic design.

The men we seek will collaborate with colleagues and with experimental teams involved in problems that require model formulation, analysis, numerical methods and computer software development.

Traditionally, a wide measure of freedom is enjoyed by research workers in our laboratory.

We believe that this engenders a fundamental approach to problems.
Members of the laboratory are encouraged to publish the results of their investigations and to contribute to international conferences.

Application letters, with curriculum vitae, references and titles of publications are requested to be sent to
N.V. Philips' Gloeilampenfabrieken
Personnel Department, Ir. H. P. Maas Geesteranus
Eindhoven, the Netherlands.

N.V. Philips' Gloeilampenfabrieken,
Personnel Department, Eindhoven, the Netherlands.

PHILIPS

(1507)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

Applications are invited for the following posts:

LECTURER/RESEARCH DEMONSTRATOR IN MICROBIOLOGY

DEPARTMENT OF BOTANY

The posts will be tenable for one year from October 1, 1970, with the possibility of renewal. Candidates in any field of Microbiology will be considered, but those with interests in Virology or Marine Microbiology may receive preference.

The salary will be within the range £1,240 to £1,585 for the Lectureship, and £1,010 to £1,065 for the Research Demonstratorship, according to age, qualifications and experience. Both posts carry F.S.S.U. benefits.

Closing date Thursday, April 30, 1970.

LECTURER IN CHEMICAL ENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

The appointment will be tenable from October 1, 1970. The salary will be on the scale £1,240 to £2,850 per annum, plus F.S.S.U. benefits.

The Head of the Department, Professor J. F. Richardson, would welcome informal enquiries from potential applicants.

Closing date Saturday, April 25, 1970.

Further particulars, quoting Ref. No. B6, may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, Glam, SA2 8PP, to whom applications should be sent by the above specified dates. (1458)

UNIVERSITY OF MANCHESTER

LECTURER IN PHYSIOLOGY

Applications are invited for this post from candidates with a good honours degree in Physiology or related subjects, or with medical qualifications registrable in this country. Appointment will be made in the range £1,240 to £1,585 per annum. F.S.S.U. where appropriate.

Particulars and application forms (returnable by May 2) from the Registrar, The University, Manchester, M13 9PL. Quote ref : 69/70/Na. (1476)

DEPARTMENT OF PHYSICS

UNIVERSITY OF MANITOBA

Offers generous assistance to highly qualified students wishing to do graduate work in physics. Extensive and modern facilities for work in nuclear structure and reactions, solid state, low temperature, atomic physics, and cosmic rays are available.

For information apply to: Head, Department of Physics, University of Manitoba, Winnipeg 19, Man. (X1310)

HAILE SELASSIE I AIR FORCE ACADEMY

Applications are invited for a post of Assistant Professorship in the Mathematics Department. Applicants will be required to teach Mathematics in the First, Second and Third Year.

QUALIFICATIONS: Holders of M.S. in Mathematics with a minimum of three years' teaching experience.

Post to be assumed September, 1970.

SALARY: Eth.$15,000 net per annum (Eth.$6 equals £1 sterling). Family passages and accommodation provided. Three-year contract, renewable if agreed upon by the two parties.

Applications and inquiries to: The Dean of Science Faculty, Haile Selassie I University, P.O. Box 1176, Addis Ababa, ETHIOPIA. (1325)

CSIRO

RESEARCH APPOINTMENT IN BIOLOGICAL CONTROL

AUSTRALIA

DIVISION OF ENTOMOLOGY

GENERAL Applications are invited for appointment as Research Scientist (Biological Control) with the Division of Entomology of the Commonwealth Scientific and Industrial Research Organization, located at Canberra, A.C.T. The Division of Entomology is the main centre in Australia for basic research on insects and is also actively engaged on many problems of economic importance; the professional staff numbers about 100. The Division's research programme ranges over the field of entomological research with particular emphasis at the present time in the fields of population dynamics and ecology, physiology and biochemistry, taxonomy, and biological control. Many studies are in progress on methods of insect control that do not rely upon the use of insecticides. There are excellent facilities for a wide range of research activities.

DUTIES The appointee will be required to carry out research within the broad field of biological control of weed and insect pests.

QUALIFICATIONS Applicants should have a Ph.D. degree or post-graduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The duration of the appointment will be determined in consultation with the successful candidate; some applicants may prefer a fixed term appointment for three years. The appointment will be conditional upon passing a medical examination and an initial probationary period of twelve months may be specified. An indefinite appointment carries with it Commonwealth Superannuation Fund or Provident Account privileges. Further details supplied on application.

Applications, (quoting Appointment No: 180/538), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64-78 Kingsway, LONDON, WC2B 6BD

by the *15th May, 1970.*

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(1551)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF CHEMICAL ENGINEERING

CHEMICAL TECHNICIAN

required, preferably with H.N.C. to work within a research group investigating polymers. The work requires an interest in analytical and preparative chemistry, and the successful candidate must be able to work without close supervision. The laboratories are modern and well equipped, and present an opportunity to specialise further and gain experience in other techniques, such as Electron Microscopy.

Salary at present under review but currently on a scale rising to £1,127 per annum.

Please apply to: Dr. G. F. Freeguard,
Department of Chemical Engineering,
The University, Nottingham

giving details of career to date and names and addresses of two referees. (1538)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF ANATOMY AND

MANCHESTER ROYAL EYE HOSPITAL

Applications invited from candidates with medical qualifications registrable in this country for two joint posts of Temporary Lecturer in Anatomy/Senior House Officer in Ophthalmology. The persons appointed will work in the University Department of Anatomy and in the Eye Hospital, spending approximately half their time on work in each department. Experience in Ophthalmology is desirable; candidates working for Primary F.R.C.S. acceptable. Salary range per annum : £1,570 to £1,790. Initial appointment for one year commencing October 1, 1970, but renewable for a second year.

Particulars and application forms (returnable by April 29) from the Registrar, The University, Manchester, M13 9PL. Quote Ref : 64/70/Na. (1477)

SK&F

PHYSICAL ORGANIC CHEMIST

Smith Kline & French Laboratories Ltd. is the U.K. Division of a leading international pharmaceutical group. Our Research Laboratories and Head Office are located in Welwyn Garden City.

A vacancy has arisen for a Ph.D. Chemist, with several years experience, to join the Physical Organic Chemistry Department of the Research Institute. The successful applicant will supervise the IR/UV Laboratory, and he will be required to work closely with our medicinal Chemists to provide physico-chemical support using a variety of techniques. Experience in the measurement of dissociation constants and partition ratios, as well as a knowledge of thermodynamics and statistical mechanics would be desirable. Some experience in the use of computers would be an added recommendation.

Fringe benefits include annual bonus, non-contributory pension and life assurance schemes, and subsidized staff restaurant. Full relocation expenses will be paid to applicants joining this successful organization.

Please write or telephone for an application form to.

C. J. Baron, Personnel Officer,
Smith Kline & French Laboratories Ltd.,
Mundells, Welwyn Garden City, Herts.
Telephone: Welwyn Garden 25111—Ext 342.

(1568)

INSTITUTE OF OCCUPATIONAL MEDICINE EDINBURGH

ERGONOMIST

A graduate ergonomist is required in the Physiology Branch of the Institute of Occupational Medicine, Edinburgh, which is associated with the University of Edinburgh.

The successful candidate will be employed by the National Coal Board but stationed at the Institute.

The successful candidate will assist a senior ergonomist at the Institute in the investigation of problems connected with the Coal Industry. Field work at various coalfields throughout the country will be undertaken.

Salary will be within ranges extending from £1,160 to £2,335 according to qualifications and experience.

Applications, stating age, qualifications, experience etc., should be sent to the **Staff Manager, National Coal Board, Lauriston House, 80 Lauriston Place, Edinburgh,** before 25th April, 1970, quoting Reference 45/70.

(1545)

AUSTRALIAN NATIONAL UNIVERSITY

INSTITUTE OF ADVANCED STUDIES THE RESEARCH SCHOOL OF PHYSICAL SCIENCES

Director: Professor Sir Ernest Titterton, C.M.G., F.A.A.

CHAIR OF APPLIED MATHEMATICS

Applications are invited for appointment as the Foundation Professor and Head of the new Department of Applied Mathematics, Research School of Physical Sciences.

The new Department of Applied Mathematics will join the six existing Departments (Astronomy, Engineering Physics, Geophysics and Geochemistry, Pure Mathematics, Nuclear Physics, Theoretical Physics) and two Units (Diffusion Research Unit and Electron and Ion Diffusion Unit) within the Research School of Physical Sciences. An eighth Department—Solid State Physics—will be established within the next year. The School of General Studies of the University has Departments of Pure Mathematics, Applied Mathematics and Theoretical Physics.

The new Department will be developed over a period of two to three years to an ultimate staffing level of twelve (including academic staff), with up to ten research students supporting its work. The Head of the Department will be responsible for choosing the staff and developing a research programme in chosen areas of Applied Mathematics. However, it is hoped that there will be a strong link between some of the Department's work and fields of experimental work already under way in Canberra, either within the University (which, in addition to Physics, has Schools of Biological, Chemical, Medical and Social Sciences) or in other research organisations such as the C.S.I.R.O.

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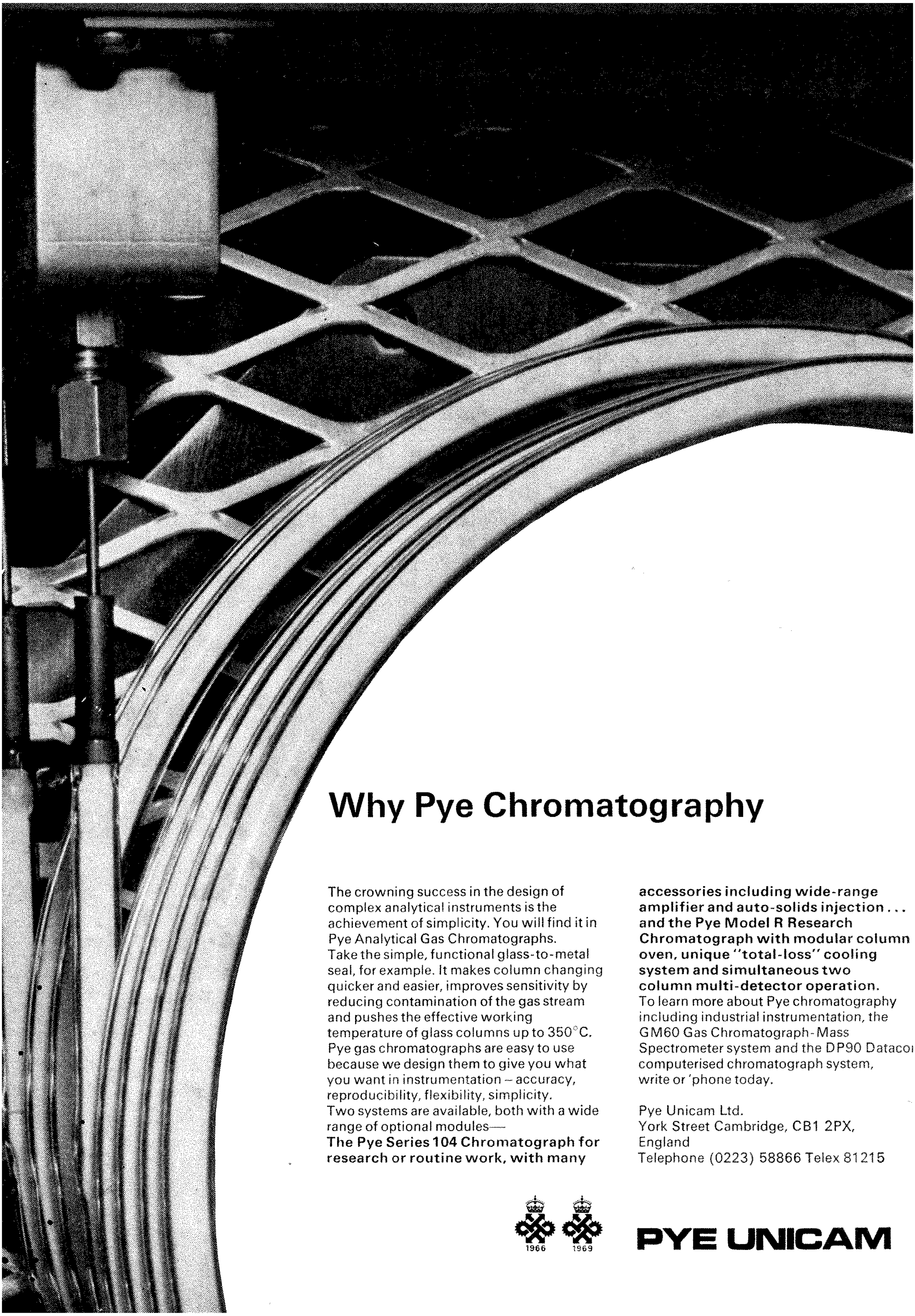

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NATURE Volume 226 APRIL 25, 1970

Is there a Future for Science in the United States?

THE foolish muddle in the United States about funds for academic science in the coming financial year is bound to leave a deep scar. Even if, against the odds, the National Science Foundation emerges by July with more money than it was allowed to ask for—$50 million more if Senator Edward Kennedy has his way (see page 307)—and even if the Administration agrees to spend the extra, there will not be enough to go round. So much is already clear. The whole of $50 million would be needed simply to keep alive the projects being dropped by the Department of Defense. There would be nothing left over to support projects abandoned by other agencies—the Princeton–Pennsylvania accelerator which the Atomic Energy Commission proposes to abandon, for example, and the projects in organic chemistry of which the National Institutes of Health have washed their hands. And, however generous Congress may think it safe to be, there is unlikely to be money to meet the needs of those who responded four or five years ago to the cry for more graduate students, and who are now ready to set up on their own. Moreover, there is no chance of funds for making good some of the glaring deficiencies which have been allowed to accumulate—the scandalous shortage of radio telescopes, for example. To be sure, it has become fashionable to say that science ought to be bent to more practical tasks, but there is very little to suggest how this can best be done and not a shred of evidence to show that damaging the splendid structure of American science which has been built up in the past 25 years will help. The issue is whether the United States shall remain the pacemaker of scientific discovery which it has become.

To worry about the budget for the next twelve months is therefore less important than to be alarmed for the more distant future. Experience in the past few years suggests that trouble about the budget of the National Science Foundation will be an annual occurrence. The most distressing feature of this year's fiasco is the Administration's indifference to the consequences of its decisions. The notion that future generations of graduate students should be supported by loans, supposedly the origin of the decision that the National Science Foundation should not make traineeship awards after this September, will have all kinds of apparently unexpected results—it will tend to concentrate graduate education in the stronger departments, and will tempt those who apply for research grants to include graduate students on their payrolls, thus further reducing the independence of students. After all the care that has been spent in past decades on the encouragement of graduate education, it is almost unthinkable that the Administration should have foisted this principle on the system in such an unreflective way. So it is time to think of structural changes that may insure against a repetition of these follies.

Where should reconstruction begin? The Federal Government must first make up its mind what it hopes to gain from its support of basic research in universities, directly through research grants and by the support of graduate students. To begin with, it has never acknowledged with sufficient generosity that federal spending is necessary if higher education is to flourish. The first report of the National Science Board in January 1969, which eloquently argued the case for a growing commitment to graduate education, has been studiously ignored. The $1,000 million or so of federal research money which finds its way directly to the universities each year is, however, an indispensable assurance of the quality of university education, undergraduate and graduate. The Federal Government has also in the past been concerned with the importance of discovery, and the way in which American universities have helped to break new ground throughout science in the years since the first Sputniks appeared is a moving illustration of the power of pure reason. Is all this to be thrown away now that the answer to the Sputniks—the flight of Apollo 11 last year—has been produced? And has the Federal Government forgotten how much the vigour of some of the mission agencies since the Second World War has depended on the work of scientists at universities? Some may think that short commons will force the goose to lay more golden eggs, but it is more likely that the treatment may prevent it from laying altogether.

From these principles follow several simple rules. First, continuity is essential. President Nixon himself was fond of saying, during his election campaign, that "you can't turn science off and on like a faucet". Discontinuity saps the morale and turns away intending scientists. It also dissipates the momentum of discovery—it is hard to turn intellectual creativity off and on. But discontinuities are also wasteful—the proposals for the coming financial year will entail a great many profligacies—the closing down of the Princeton accelerator is but one illustration of the wanton discarding of valuable resources.

The second rule which must somehow be accepted both by the Administration and by Congress is that the scale of support for university research should be determined partly by decisions about the scale and quality of higher education and partly by judgments about the development of science as such. If, for example, the objective is to improve the standing of the weaker universities, ways of geographically spreading out research funds should be sought. If there seems to be a need to encourage research in a new field —teratology, perhaps, with all the excitement about the potentially harmful consequences of food additives —then pump-priming must be a public expense.

It also follows that in the allocation of research money to different areas of research, a balance should be struck on scientific and not administrative grounds. One of the defects of the present arrangements is that large parts of the spending on basic research are not subjected to the thorough analysis which, for housekeeping reasons, the National Science Foundation and the National Institutes of Health consider necessary. High energy physics is isolated and insulated beneath the umbrella of the Atomic Energy Commission. Much of what American universities do in materials science —there is probably too little of it—is supported by the Department of Defense. And, however great the diligence with which the National Aeronautics and Space Administration sets out to examine proposals for research in astronomy and the physics of the solar system, these are not weighed against more modest land-based proposals intended to accomplish the same ends. Thus it is doubtful whether NASA would have been about to build the X-ray telescope due to be launched in the first manned astronomical satellite if it had had to apply to the National Science Foundation for the money. Anomalies like this are by themselves sufficient cause for welcoming the spirit of the Mansfield amendment to the current defence appropriation— the principle should be that the mission agencies support basic research only when there are special reasons why this is essential. In short, it is now time to abandon the plurality of agencies which, in the fifties and sixties, gave scientists the illusion that there was no shortage of money.

A further corollary is that there should be a greater degree of independence for whatever agencies are responsible for spending federal money on scientific research and higher education. To be sure, in the last resort the government must decide how much money there is to spend—that is inescapable. But the people responsible should then be free to use the intricate system of advisory committees which they have developed to decide precisely how funds shall be allocated. Although the National Science Foundation has the theoretical benefit of not having to justify its budget item by item to Congress, the Bureau of the Budget seems to use a remarkably fine-tooth comb. But why should the Administration keep a dog and then bark itself (through the Bureau of the Budget) ? It would be better for everybody's sake, for example, if the National Science Foundation were to decide whether to pay stipends to graduate students directly or let them be added to research grants. And why should external influences be so powerful in deciding how much is spent on radio telescopes ? One of the defects of the present interaction between the Administration and the agencies responsible for science is that the little money there is may be spent less well than possible. Another is that the agencies concerned do not develop the general concern for the forward planning of the scientific enterprise that should be central to their functions. Then the general sense of fickleness which runs through a great deal of what passes for science policy serves powerfully to discourage those who do the donkey work on the advisory committees and their constituents.

So what is to be done ? The best hope is that the new Administration's concern for organization will provide an opportunity for putting the financing of scientific research on a more secure foundation. The Ash Commission will be making recommendations to the President throughout this year—proposals on the agencies concerned with the environment will probably go forward before the end of April, for example. Although there are some who say that the problems of the National Science Foundation would disappear if only its budget were twice as large, mere money will not avoid the structural defects in the present system. To be sure, the National Science Foundation should be at the centre of any reorganization, but the objective should be to add not to its budget but to its responsibilities. Thus it would be sensible to transfer from the Atomic Energy Commission responsibility for high-energy physics, together with some of the laboratories involved. Some parts of the work done by the National Institutes of Health should also properly belong to an agency concerned with the support of university research. And there is a case for channelling external advice on the expenditure of NASA and the DOD on university research through the same advisory committees.

Simple repackaging, however, is not enough—it is also necessary to provide a stronger agency with a self-renewing sense of purpose. Is it entirely beyond reason that there should be an organization with broad responsibility for the development of the understanding and the skill which the modern world increasingly demands ? This, after all, is what the National Science Foundation has been trying to do on its perpetual shoestring. The National Institutes of Health have done equally valiant work in medical education. Research, training and curriculum development have gone hand in hand. It may now be opportune to broaden the scope of this work. And this is a cause for which a properly chosen board would be able to fight coherently—and publicly. To be sure, there are many congressional committees and interests outside Congress that would resist the notion, but that is no reason for doing nothing.

In these discussions, the Office of Science and Technology seems increasingly irrelevant. That may be inevitable, for the days are probably gone when the presence of a scientific adviser at the White House could give the scientific community a sense of being properly represented at the centre of power. For one thing, times have changed. The scientific community as such is now less well placed to give crucial advice on urgent problems than in the fifties; it can expect fewer favours in return. But the office was lent special lustre by those who first occupied it and by their personal influence with the president of the day. It is no longer safe to rely on the good chance that the incumbent will be at once able and influential, but in any case the prestige of the office has in the past two years been severely damaged by the trouble over the budget of the National Science Foundation. In the circumstances, it would be better for the scientific community as a whole that there should be a strong organization built around the National Science Foundation, and free to speak out when policies seem misconceived, than that there should be an Office of Science and Technology at the White House. That could be a valuable counter in any bargaining on organization there may be.

Pull Up More Weeds

THE events leading to the decision last week in the United States to restrict the use of the herbicide 2,4,5-T, reported in their tedious detail on page 309, embody important lessons for those anxious to see emerge more deliberate procedures for the regulation of chemicals in the environment. From this point of view, the notoriety that 2,4,5-T has acquired through its use in the attempted defoliation of large areas of Vietnam is almost irrelevant, even though there will be some relief that the United States Army will now restrict the use of 2,4,5-T (but not necessarily of the other herbicides used for defoliation). The case against the use of herbicides for defoliation has quite properly been based on the doubt which there must be about the ecological damage which may be done by large-scale destruction of vegetation. Even hawks should be asking what purpose would be served by winning the war by methods which might make it necessary to wait for decades to see the land recover its capacity to bear crops. In narrower terms, there are also serious doubts about the tactical effectiveness of defoliation that should long since have given the US Army pause in its policy of defoliation—is a farmer whose crops have been destroyed more, or less, likely to join the Viet Cong ? The implications of questions like these for what happens in Vietnam remain important, but they are separate from the issues now raised by the restrictions on the civil uses of 2,4,5-T.

It is only fair to acknowledge that a good deal of present concern for the biological consequences of trace amounts of organic chemicals in the environment stems almost directly from the way in which chromatography in its several versions has made possible the detection of some of these materials. To say that is not to complain that chromatography has made life more difficult, of course, but merely to suggest that a new range of problems has been brought within reach of tackling. It is also proper to admit that several recent scares have drawn attention to previously unsuspected sources of biological hazard—the thalidomide incidents in Britain and Germany have cast a long shadow. What is happening now, belatedly perhaps, is that regulatory authorities are being forced, either by their own inclinations or by pressure from outside, to re-examine old decisions to permit the public use of various chemicals, food additives, herbicides, insecticides and the like. That such a process is under way should not be regarded as a sign that strange chemicals now assault the environment to such a dangerous extent that extraordinary steps have to be taken to avoid trouble—it is equally fair to say that opportunities have now been provided that will ensure a safer environment. The way in which specific hazards of some kinds of factory employment have been eliminated in the past half century by formal regulation is a proof that the process is continuous. It would be in everybody's interest if there could be a more common understanding of how these matters should be organized.

First, panic and the appearance of panic can and should be avoided. The way in which the Secretary for Health, Education and Welfare in the United States decided last October to take cyclamates off the market has diminished the credibility of those public officials whose job it is to regulate chemical hazards. The circumstances leading to the banning last week of the herbicide 2,4,5-T are not much better. Everybody knows that experimental data whose significance (in the strict sense) is worked out over the weekend tend to be made too much of. As things are, nobody can be quite sure what weight to give to the different pieces of evidence upon which the decision to restrict the use of 2,4,5-T is based. It looks as if the experiments which have been carried out show that in mice, but not in rats, pure 2,4,5-T is teratogenic. Everybody would wish, however, for more extensive experiment and for more time to consider the results. The fact that there is at present a sense among politicians that the public wants quick action should not blind them to the need that this action should be deliberate. The way in which the Department of Health, Education and Welfare has had to beat a partial retreat from its original position on cyclamates should have been a reminder of what may happen when decisions are made too quickly.

But how are decisions about the safety of chemical contaminants to be arrived at ? The present convention is that the politician responsible for the appropriate department of state, advised by his officials, will shoulder this responsibility. The trouble here is that the data frequently do not lend themselves to unambiguous interpretation. Deciding what to make of a series of unrelated animal experiments with unfamiliar chemicals in small quantities is a little like the familiar problem in the physical sciences of trying to decide which of several alternative values for a constant of nature is most to be trusted. In those relatively simple circumstances, decisions are hard to make and time-consuming. Moreover, the process is one of scholarship, not political decision-making, so is there not a case for letting the crucial assessment of the risks involved be decided by something like the familiar academic process in which a group of people worries out what seems to be the truth and then lets the rest of the scientific community say whether the diagnosis makes sense? The politicians may say that the public will not wait that long, but this is surely mistaken. There is no evidence that the imperfectly assessed hazards of chemicals recently thought safe are so conspicuous that damage will be done if decisions are postponed for a few months.

Assessments like these will, of course, take time and skill at present in short supply, and one of the most striking lessons of the public inquiry into the use of 2,4,5-T is that there is an urgent need for more professional research of a kind which can help to define teratological and mutagenic hazards. Since in the long run the best hope is that better understanding in embryology and carcinogenesis will help to identify trouble in advance, the emphasis should be on university research in collaboration with more fundamental studies, and not on the attempt to win quick answers by animal experiments blindly carried out. This is an urgent need, and there is, unhappily, very little sign that the governments anxious to respond to popular feeling about contaminating chemicals are prepared to spend the trouble and the money.

The implications of a more active policy by governments towards contaminating chemicals for the relationship between governments and industry are potentially important and should also be thought of in advance. The Mrak commission on pesticides in the United States estimated last year that the cost of bringing a new agricultural chemical to the market is about $5 million, and it is plain that manufacturers will be less willing to spend money on research and development if the chances of being able to market an otherwise successful product are restricted by the discovery of some unwelcome and possibly unexpected side effect. Moreover, it is anomalous that most governments—certainly the British and the American—should expect manufacturers to pay for the cost of the tests necessary to demonstrate that a new chemical can be safely used. So is there not a case for asking that some part of the increasingly onerous cost of testing should be borne by the central government? This is not so much a matter of equity as of making sure that the inventiveness of the chemical industry is not stifled to a degree that will limit its social value. To be sure, where proprietary materials are involved, other issues of principle arise. Governments might find it necessary to ask for a transferable patent right for compounds tested with public money. Whatever the pitfalls, however, governments wishing to claim a good reputation on environmental chemicals had better face up to problems like these.

Should governments, however, operate independently? It is conventional to pay lip service to the view that international collaboration is essential. In practice, precious little has been done. Even in the exchange of information about chemicals in the environment, good intentions are victims of haste like that which led to the restriction on 2,4,5-T last week. The truth seems to be that governments and their officials do not believe that international collaboration has anything of value to offer. The Mrak commission which reported in the United States last December was in general a monument of good sense, which nevertheless finished its lukewarm recommendation on international action with the meaningless suggestion that "the US government should assume leadership in studying the inherent health hazards of pesticide usage and cooperate in the training of technical personnel from other countries". Is this the best that an otherwise enlightened committee can suggest? Is there some reason why the process of testing the safety of novel compounds cannot literally be shared within the scientific community? Why should not organizations such as the World Health Organization play a part in the administration of these schemes? For all the drama of the announcement about herbicides last week, the wisdom of the decision taken remains in doubt—it may yet turn out that the Dow Company's case is upheld by further work—but the need for better machinery has been demonstrated for all to see.

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100 Years Ago

It is no longer a secret, that wherever a persistent warfare is carried on against small birds—against martins, blackbirds, sparrows, larks, &c.—vegetable life is sure to suffer. In the Isle of Bourbon, as M. Michelet tells us, the martin was exterminated, and a plague of grasshoppers followed; in Hungary, the sparrow was proscribed, until this valiant militia of the fields had to be recalled; in the neighbourhood of Rouen, the blackbird was shot down without mercy, and many a meadow's turf could be rolled up like a carpet; and in this country we had, until lately, our sparrow clubs, which paid for little victims at so many a dozen, just as two or three hundred years ago some of our pious churchwardens used to purchase hedgehogs of truant schoolboys in the rural districts, a lamentable increase of pestiferous insects being the consequence. The Rev. Charles Kingsley gives us a more recent example. In Trinidad, the free negro has been banging away at the small birds, partly for his own pleasure, and partly to supply the London markets and our ladies' hats. What has been the consequence? "Already the turf of the savannah, or public park, close by, is being destroyed by hordes of mole-crickets, almost exactly like (strange to say) those of our old English meadows; and unless something is done to save the birds, the canes and other crops will surely suffer in their turn.

From Nature, **1**, 648, *April* 28, 1870.

OLD WORLD

MOLECULAR BIOLOGY

EMBO goes Critical

Molecular biology in Europe has just taken another step forward with the establishment of the European Conference on Molecular Biology. Twelve states (Austria, Denmark, France, Greece, Italy, Netherlands, Norway, Spain, Sweden, Switzerland, Britain and West Germany) have already joined the organization; Israel is also to be a member and the Fonds National Belge will assume the obligations which would have fallen on Belgium had it joined. Financial contributions from member states will be assessed on the basis of net national revenue. The budget for 1970 amounts to $632,000 and expenditure for 1971 is planned to be about $1 million. EMBO, which has hitherto been supported largely by the Volkswagen foundation, will now receive its funds from the ECMB.

The conference is normally to meet once each year, but because it has only just been established and the first plenary session held, it is due to meet again in November. The project to establish a European Laboratory for Molecular Biology is gathering momentum, and EMBO has recently revised its earlier proposals. The ECMB has set up a working group to study the revised proposals, and this is to meet in September and report to the subsequent meeting of the ECMB.

The underlying philosophy of the EMBO proposals remains largely unaltered. There will be only a small number of permanent scientific staff; the laboratory will place strong emphasis on the advanced teaching of postdoctoral fellows by conducting courses and workshops after the example of Cold Spring Harbor in the United States; and there will be "project space" available for groups which wish to use special facilities for particular experiments. The plan differs, however, in emphasis and in scope from its predecessor. The first plan was to establish a laboratory which would grow over a period of eight years to about 260 scientists and costing some $18 million. The laboratory would have an annual budget of about $10 million. The revised plan is more modest, and demands only an initial laboratory of about sixty scientists, with an initial cost of about $5 million and an annual budget of $4 million. Questions of expansion are left to future negotiations.

Rather more detailed proposals are now made for the research programme, although EMBO acknowledges that these are examples of the nature of the research which may be carried out rather than firm intentions. Both the present trends of molecular biology are reflected in these suggestions. The reductionist approach which aims to explain biological functions in terms of the physicochemical properties of molecules is represented by proposals which include the study of macromolecular assemblies such as membranes and ribosomes. The second approach, the application of the concepts and methods of molecular biology to more complex systems, is shown in proposals for research into the cell genetics of mouse and man and for a genetic study of the structure and function of membranes. The laboratory would also have as a major task the development of the sophisticated instrumentation which is likely to be necessary for future experiments in molecular biology.

The report of the ECMB working group on these proposals is the critical stage in the development of an EMBO laboratory. The omens are good that the group will agree on the establishment of such a laboratory, for of the fourteen countries of the ECMB, eleven have expressed general approval and only three remain neutral. There remains the danger, however, that if agreement is reached, there will be dispute as to which country is to house the laboratory. EMBO itself has suggested Geneva, where CERN is already located, and offers of sites have come from Austria, France and Italy. Other countries are also rumoured to be considering making bids to acquire the laboratory. But even if full agreement is reached in November, it will still be some considerable time before a laboratory is established. Design and building are likely to take at least three years, so that the laboratory would not be able to commence its activities before 1973 and would not be fully functional until perhaps 1975. A glimmer of hope for an earlier establishment, however, may be found in the suggestion that temporary accommodation may be sought to enable an EMBO laboratory to start work within the next couple of years.

PARTICLE ACCELERATORS

Sights Lowered at CERN

Just as hope was receding that the 300 GeV accelerator project would ever get off the ground, an entirely new plan was put forward by Dr Adams's group at CERN last week which seems to have most of the ingredients of a working compromise. The idea is to start off by building a machine to produce particles of 150 GeV, but to use what is known as the "missing magnet" design to allow the maximum energy to be raised at a later date to 300 GeV or 600 GeV, as physics and the economic circumstances dictate. The initial outlay for the 150 GeV accelerator would be a third less than the £150 million budgeted for the former 300 GeV project.

In the new proposals, which seem to be intended as a sort of CERN green paper, the ring of 2·4 km diameter suggested for the old 300 GeV scheme would be replaced by one of 1·8 km diameter which could be fitted into the existing CERN-Meyrin site near Geneva. This would avoid the wrangle over a site which jeopardized the earlier project. If superconducting magnets become a practical reality for synchrotrons within the next ten years, as many people believe, additional magnets could then be added to the ring and the conventional magnets replaced to produce fields capable of accelerating protons up to 600 GeV or even 800 GeV.

In the light of the new proposals, it seems that the way the original 300 GeV scheme was formulated may have been a tactical error. For one thing, it left little scope for incorporating significant advances in magnet technology and it presented governments with a single investment decision of considerable magnitude. To be sure, the advantage of a fixed energy machine is that the design can be optimized throughout for the peak energy, but with so many other variables in the equation this may well have been too blinkered an approach.

Of the three types of accelerator that Dr Adams's group could have considered, the missing magnet design is in a sense the obvious choice. It is the most technologically flexible, which is important if the machine is to be extended in energy beyond the limit of the 200 GeV Batavia accelerator in the United States, which is already nearing completion.

Moreover, the decision to boost the maximum energy by a factor of four or more can be left to depend on the results of the first round of experiments. In the "missing power" type of machine as at Batavia or the fixed energy type as in the original CERN proposals the options are far more limited, although the Batavia accelerator can, of course, be extended up to 400 GeV if required.

The inspiration for the new proposals no doubt derived from the rapid progress in developing superconducting magnets, notably at the Rutherford High Energy Laboratory in Berkshire.

GENERATORS

Breakdown in Detail

LACK of attention to detail in the design of 500 MW generating units was largely responsible for the plant breakdowns that led to voltage reductions last December. This verdict of the Select Committee on Science and Technology is clearly spelled out in the committee's first report to Parliament this session (HMSO, 20p) and follows chiefly from evidence given to the Select Committee by representatives of the Central Electricity Generating Board (see *Nature*, **225**, 496; 1970). Other faults exposed in the report are hairline cracks and defective welds in the boilers and "malicious damage".

The Select Committee began its investigation after the Minister of Technology had said in a written reply that he thought an official inquiry would not help. But Mr Arthur Palmer's committee decided that a brief fact-finding inquiry was in order and, although the report can find little room for major recommendations, it tells a sorry tale of annoying faults that eventually caused sixteen 500 MW generators to fail, and reduced power in the grid on five separate occasions.

The only remedies that the committee can suggest are that the CEGB should place design contracts with boilermakers before the final contracts for steam-raising plant are finalized, and that every effort should be made to test plant before it actually generates electricity into the national grid. Placing design contracts in this manner will, in the committee's opinion, encourage the setting up of strong design teams, who could be called on whenever precise data are needed for development work. As far as testing is concerned, however, the committee recognizes that little can be done to test large components on full load before they are installed in power stations, but it welcomes the plans for wider testing of the 660 MW sets which are now being developed.

Evidence submitted to the Select Committee by Mr E. S. Booth, the CEGB's member for engineering, also pointed to the possibility that some of the failures were caused by tools and other foreign bodies being deliberately left in vital parts of equipment. But both he and Sir Stanley Brown, chairman of the CEGB, said that site discipline is an intractable problem and, although methods have now been devised for detecting foreign bodies in plant, little can be done to prevent such occurrences.

The other major headache of the CEGB—corrosion of mild steel nuts and bolts in magnox reactors—also came under the Select Committee's scrutiny. It seems that the operating temperatures have now been reduced in all the affected reactors, and output has been cut back by a total of about 500 MW. But despite these setbacks, the reactors should enjoy at least their economic life. The committee believes, with the benefit of hindsight, that nuclear engineering teams need strengthening by people interested in corrosion problems of all kinds, and with the technical competence to handle them, and it also recommends that all items of equipment whose corrosion properties cannot be predicted with certainty should be removable.

HOVERTRAIN

New Track for Roskill

A NEW aspect to the discussion of where best to site London's third airport has been introduced by the recent disclosure that tests on a hovertrain capable of 300 miles per hour are to begin later this year on an experimental track just north of Cambridge. If successful, this would mean that an airport anywhere within a hundred mile radius of London could be reached in less than half an hour from the centre of the city. This may be the signal to banish the new airport once and for all from the heavily populated areas around London.

Engineers at Tracked Hovercraft Ltd, a subsidiary of the National Research Development Corporation, expect to carry out the first trial runs at the end of October 1970. The first three miles of track should be completed within the next two months and a further four miles will be constructed by the end of next year to permit the test vehicles to reach their cruising speed of 250 miles per hour.

The Roskill Commission investigating the question of London's third airport may now wish to extend the scope of its inquiry to include new technological developments. Tracked Hovercraft points out that the site at Foulness could be linked to the centre of London by a 25 minute hovertrain service to King's Cross, shuttling 100 passengers every two minutes in vehicles about the size of a standard railway carriage. The hovertrain would be driven by a linear induction motor, which is preferred to the French propeller driven model on the grounds of being quieter and more versatile.

Tests on the French hovertrain are already in progress at Orléans. With the foundations being laid now for a third airport at Paris, to the north of the city, the talk of a possible hovertrain link to the centre of Paris seems more than a flight of fancy. But although the technical feasibility of air cushion transportation is already well established, commercial viability seems to be a tougher proposition. The British Hovercraft Corporation, which makes the 150 ton SRN4 hovercraft, announced last week that it would be closing its factory at Itchen, Southampton, for lack of orders. The fifth model of the SRN4 is half complete and seems to be without a prospective buyer. Work on the smaller SRN6 and the 40 ton BH7 will be transferred to the company's main plant at Cowes.

GEODESY

Life without Echos

GEODESISTS are finding it hard going without the once familiar glow of the Echo satellites to illuminate their triangulations. Echo 1 re-entered the atmosphere on May 24, 1968, and Echo 2 followed a fraction more than a year later on June 7, 1969. During their lifetimes the Echo satellites—strongly reflecting balloons 41 metres in diameter—became well known around the world and, even, it is rumoured, venerated as gods in the African bush. With apparent magnitudes on the stellar scale of about 2·5, the satellites could easily be detected by the small cameras of the Western Europe satellite triangulation programme. (The larger the magnitude number the fainter is the object, and the limit for the eye is about magnitude six.) The aim of the programme is to obtain accurate locations for a number of stations in Europe relative to each other by photographing satellites against the background of stars. Geodesists are now having to make do with less bright satellites, and the situation was discussed at the Royal Society last week in a meeting of a subcommission of the International Association of Geodesy which deals with observations of artificial satellites from Western Europe.

As well as pressing for a third Echo satellite, the meeting appears to have backed another horse: the provision of more cameras able to track the faint satellites. Two of the balloon satellites which are at present being used for the programme—Explorers 19 and 39—are frequently too faint for the majority of European cameras. Last year's meeting of the subcommission in Paris initiated a test programme of observations of Explorer 39 beginning in May last year, and so far it appears that only five European countries have been able to obtain successful photographs. The reason is that the Explorer 39 balloon is only a few metres in diameter and that it is in an elliptical orbit so that its brightness, at best of magnitude 3·7, is variable.

A third balloon, the Pageos satellite consisting of a 30 metre diameter balloon of aluminized Mylar, is more easily visible at magnitude 1·6, but its orbit at a height of 4,000 km is less good for triangulation work over short European baselines than satellites at lower altitudes.

The subcommission is also looking forward to the launch of further satellites equipped with reflectors so that the more precise technique of laser ranging can be used. The next satellite in the series is Geos-C, expected to be launched in 1972, and the subcommission wants to see the laser method developed and used at European stations. At present satellite triangulation methods are giving accuracies in position of the order of 30 metres, less accurate than classical methods, but the hope is to improve the accuracy to about 3 metres using the standard photographic method, and to less than one metre using lasers.

But the subcommission is still hopeful of seeing another balloon satellite of the Echo type in a low altitude orbit, especially now that more countries are joining the programme with less powerful cameras.

Thirteen countries are participating in the triangulation programme with more than thirty cameras. In Britain, stations are at Malvern, operated by the Ordnance Survey, and at Earlyburn, near Edinburgh, operated by the Royal Observatory, Edinburgh. Both stations have successfully photographed Explorer 39 down to fainter than magnitude seven.

PRIVATE RESEARCH

Arthur D. Little's New Name

IN the long run the government's plan to set up a British Research and Development Corporation out of a handful of the research and development organizations of the Ministry of Technology may help the independent research institutes, according to Dr F. Neville Woodward, director of Inveresk Research International. Inveresk Research International is the new name for the Arthur D. Little Research Institute which has been operating since 1956 in laboratories near Edinburgh as an offshoot of Arthur D. Little Inc., of Cambridge, Massachusetts. The change comes about because the institute has bought out its financial involvement with the parent to become a wholly British-owned organization.

Announcing this in London last week, Dr Woodward said that the independent research institutes in Britain have nothing to fear if the proposed British Research and Development Corporation is set up. Although the corporation will be competing with the independent institutes for contract work from industry, Dr Woodward thought that in the long term the encouragement of sponsored research would be a good thing. The government plan, already criticized from several quarters, is that by 1975 the proposed corporation will be self-supporting with payments for work being done for industry accounting for one-third of the annual costs. Based on the Ministry of Technology estimates, this means that the present £10 million per annum earnings from industry by the government organizations involved in the plan will have to be doubled by 1975.

At present, Dr Woodward said, "the government is moving heaven and earth to find work for its employees in laboratories which we think ought to be run down". This is one of the reasons why Inveresk Research International, which specializes in pharmacology, polymers, membrane technology and organic semiconductors, now has no contracts with government departments. But Dr Woodward also said that the staff of the institute are not enthusiastic about taking on government work because the negotiations are frequently "painfully slow", and because the institute is often not told what becomes of the work which they have done. "It's like working in a vacuum," Dr Woodward said, "so we are far less enthusiastic about government work than we were in the past." In fact, roughly half of the income of Inveresk Research International comes from the pharmaceutical industry and twenty per cent of its work is for companies outside Britain.

COMPUTER POLICY

Choice of Hardware Too Wide

THE government should not contribute to the development of new computing machinery unless what emerges is a completely different type of computer which would fit in more closely with everyday work, the subcommittee of the Select Committee on Science and Technology, which is discussing how the British computer industry can best be encouraged, was told last week by Computeraid Ltd, a British computer service bureau. Last week the subcommittee heard evidence from Computeraid Ltd and from International Computing Services Ltd, a subsidiary of ICL.

In a memorandum the Computeraid representatives talked of the embarrassingly wide choice of computer hardware, and said that the proliferation of new hardware should be discouraged. Many small and medium-sized companies do not need the facilities of the third generation of computers, and the cost of the change-over from one generation of computers to another is extremely high. It may be that the government should set up disincentives to the introduction of new computers which do not show marked improvements over previous machines, the memorandum said.

Both organizations complained about the impact on the service bureaux of selective employment tax—the payroll tax which is designed to encourage the movement of labour to manufacturing industries by operating against the service industries. The Computeraid representatives thought that software houses and computer service organizations which might be expected to improve industrial efficiency ought not to be penalized by the selective employment tax. This view was supported by the memorandum from International Computing Services, which also agreed with Computeraid that the provision of computer programs through bureaux should also qualify for investment grants.

Following the attitude of the software houses at last week's meetings of the subcommittee, Computeraid thought that the government should look beyond its internal resources for computer services, as the United States government does, and that the government should consider asking outside organizations to run government computing facilities.

The service bureaux have also been pressing the Post Office to improve the data transmission facilities which are provided through the telephone system. The Computeraid memorandum said that unless the facilities provided by the Post Office are improved the use of on-line computing is unlikely to develop to its potential. The telephone system is, of course, optimized for speech, and in the opinion of Computeraid not enough effort is being devoted to networks suitable for data transmission. The bureaux are also concerned that the Post Office monopoly may interfere with the development of data communications.

POLLUTION

Fire without Smoke

FINANCIAL coercion and governmental control are the keynote of fifty proposals for attacking pollution in Britain, put forward this week in a report from the research department of the Labour Party. The measures suggested in this report cover the setting up of three new national agencies to deal with water supplies and sewage disposal, the dumping of wastes in the sea and the reclaiming of derelict land, and a plethora of taxes, levies and fines to put pressure on recalcitrant offenders, as well as a comprehensive family planning service under the National Health Service to grapple with what is the most basic problem of all. Those who argue that the cost to industry of implementing these schemes would be economically damaging ignore both the hidden costs of pollution and the fact that other countries face the same situation, according to the report (*Pollution and Our Environment*, 3*s*, from Transport House, London SW1).

The report, prepared for the National Conference of Labour Women to be held next month in Hastings, concentrates on declaring priorities rather than planning in detail. Its credo is that, if looking after the Earth is to be regarded as a social service, then the manufacturer and the consumer must accept the need to pay up just as they do for the other social services, whether the cost be direct (effluent treatment and the like) or indirect (the increased prices that will follow). "Pollution levies", too, could persuade people to buy or sell less damaging products, as in the case of household bituminous coal, which is claimed to account for 85 per cent of the smoke pollution in Britain; a price advantage for smokeless fuels would combine fruitfully with the stronger enforcement of the Clean Air Acts which the report also urges. But industry would have to take the brunt of the cost, helped on its way by an increase in the penalties for negligence, beyond the derisory fines which are often imposed now. Prosecutions for oil spillage off British shores in 1968, for example, resulted in fines that averaged £145.

A forceful argument for clean air is embodied in a retrospective survey from the Royal College of Physicians (*Air Pollution and Health*, Pitman, London, 30*s*). In 1952 some 4,000 people may have died because of a five-day London smog; the Clean Air Act of 1956 was a direct consequence, but while the air in London is now much healthier there are plenty of northern mining towns where smoke control has not even made a start. The incidence of bronchitis is strikingly correlated with the degree of atmospheric pollution, and bad air aggravates the harm caused by smoking cigarettes (see table).

What is less clear is the relative importance of different pollutants. Smoke particles and sulphur dioxide are the worst offenders in terms of atmospheric concentrations, but until the full effect of smokeless zones has been realized, nobody can establish how much illness the two pollutants may cause separately. Carbon monoxide and lead from motor exhausts, the survey finds, do not reach obviously poisonous concentrations even in the most crowded traffic, but there is some evidence that small concentrations of

INCIDENCE OF BRONCHITIS IN RELATION TO SMOKING AND AIR POLLUTION (DATA OF P. M. LAMBERT AND D. D. REID)

	Standardized morbidity ratio* (per cent)			
	Very low pollution	Low pollution	Moderate pollution	High pollution
Non-smokers	41	52	67	71
Smokers	121	131	159	209

* Based on 100 per cent for incidence of chronic bronchitic symptoms in Britain as a whole.

carbon monoxide can interfere with concentration and perception, so that a loss of efficiency may be hidden behind the lack of overt morbidity.

Naturally enough, the survey's chief recommendation is simply to get rid of smoke, an aim that can be achieved to a high degree by outlawing the domestic coal fire. Meanwhile research should be directed towards avoiding the damage from the gaseous pollutants, ideally by reducing the sulphur content of fuels, and towards discovering their more subtle interactions with vehicle exhausts and tobacco smoke.

BULGARIA

Homage to St Cyril

by our Soviet Correspondent

THE recent announcement that the Bulgarian government has awarded Academician M. Keldysh, president of the Soviet Academy of Sciences, the Order of Cyril and Methodius (1st Class) for his services to science, seems somewhat symbolic of the whole background of the Bulgarian Academy of Sciences, which is now celebrating its centenary. On the one hand, there is the long academic tradition, symbolized by the Order named after the ninth-century "Apostles of the Slavs", who evangelized Bulgaria and formulated the Cyrillic alphabet, and on the other, the strong ties of modern Bulgarian scholarship with Russian traditions.

For, although founded in émigré conditions (Bulgaria being then under Ottoman rule), without the royal or governmental patronage under which such academies are normally born, the Bulgarian Literary Society (as it was then known) early established close and continuous links with the scientific establishments of the then Russian empire, notably with the Imperial Academy of Sciences of St Petersburg (now the Academy of Sciences of the USSR). And, over the last twenty-five years, the Bulgarian Academy has been remodelled. In the words of its vice-president, Academician Vladimir Georgiev (in his centenary address), it became "an institution of a socialist type, with specialized institutes and laboratories, which greatly broadened its field of activity".

The Bulgarian Academy of Sciences, in fact, now comprises ten departments, some fifty research institutes, as well as a number of other specialized centres, a number of which have been endowed by the Soviet Union which, for example, built the atomic pile at the Institute of Physics. The publications of the academy number sixty-three original journals and serial publications and three abstract bulletins.

In spite of its close links with the academies of the Soviet Union and other Eastern European countries, the Bulgarian Academy of Sciences has established several distinctive fields of research. The Institute for Organic Chemistry has recently obtained some most interesting results on the structure of new alkaloids isolated from Bulgarian-grown plants. The Division of Metallurgical Science and the Technology of Metals is engaged on a vast R and D project on the development and application of new alloys, based on the metals already produced by the Bulgarian metallurgical industry and the mineral resources of Bulgaria. The considerable bias placed by the Geophysical Institute on research in seismology is not unconnected with the fact that Bulgaria lies in the Balkan seismic zone.

Yet, in spite of this bias towards national conditions and problems, the Bulgarian Academy of Sciences has progressed far beyond its original aim of "disseminating general knowledge among the Bulgarian people and showing them the path to prosperity". Recent achievements of international significance include the formulation of new algorithmic languages (notably ALGAMS), the development of the "ferrograph" device for measuring the rectangular hysteresis curves of ferrite cores, the development of apparatus for measuring electron paramagnetic resonance, a new and more sensitive method for the rapid registration of the mass numbers of gases and vapours *in vacuo*, investigation of the D-layer of the ionosphere, chemistry of monomers and high polymers, microminiaturization of electronics and integrated circuits and other topics of contemporary interest.

The centenary celebrations of the academy began in October 1969, and will continue through the academic year, concluding with an international psychological conference to be held in the Black Sea resort town of Varna in September.

WATER RESOURCES

More Reservoirs for the North

A BARRAGE across the Solway Firth for estuarine storage of water will not be an economic proposition in the foreseeable future, according to the Water Resources Board (*Sixth Annual Report of the Water Resources Board*, HMSO, 10*s*; *Water Resources in the North*, HMSO, £1 16*s*). Instead, water supplies for the north-east of England should be met adequately during the next thirty years by one or two additional inland reservoirs which, the board says, will be nearer the centres of demand, and will be more acceptable aesthetically than a construction in the Solway Firth.

To meet the demands for water in the rest of the north of England in the period 1981 to 2001, the board suggests that the choice lies between more inland reservoirs (four or five), and water storage in Morecambe Bay or the Dee Estuary, or both. But this choice, it says, must be made in 1971 or 1972, and it urges that there should be further investigations soon of inland sites suitable for reservoirs. Two or three such sites may be required as early as 1976 or 1977, but the board does not see its scheme for augmenting water resources in the north becoming fully operative until 1981 at the earliest. The final choice will, of course, depend to a large extent on the results of the Morecambe Bay and Dee Estuary studies. Both studies are past the half-way stage, and final reports are expected in 1971. Progress on the third barrage being considered by the board, that across the Wash in the east of England, is not as advanced, but the board hopes to receive shortly the consulting engineers' report on the desk study which has been under investigation since 1968.

While the board believes that estuarine barrages and more inland reservoirs are necessary to meet all the future demands for water in England and Wales, much more will depend, it says, on the proper management of water after it has been used. The board would therefore like to see the sewage authorities reduced in number and made more efficient, their work planned and coordinated on a national scale, and the board's functions extended to cover the disposal of water after use so that it could have control over all aspects of water management.

NEW UNIVERSITY

Campus in Tropical Australia

from a Correspondent

ONE of the events marking the bicentenary of Captain Cook's discovery of eastern Australia is the opening of a university which bears his name. The bill setting up the James Cook University of North Queensland received royal assent during the Queen's visit on April 20. The new university was previously the University College of Townsville, part of the University of Queensland, Brisbane, promoted to full status after nine years. Situated on the Coral Sea coast near the Great Barrier Reef, the university at Townsville is the first in tropical Australia. The vice-chancellor is Dr K. J. C. Black, who was appointed warden of the college in 1963.

All facilities are being transferred from the present two campuses to a 650 acre parkland site in a natural amphitheatre bounded by the Mount Stuart Range. Most of the students, who number more than 1,200, are accommodated in residential colleges in the new setting. This year there are twenty-five per cent more matriculants than last year, a trend which is expected to continue.

This year ordinary and honours bachelors and higher degree courses will be offered in faculties of arts, commerce and economics, education, engineering and science. There are also provisions for candidates for other degrees at the University of Queensland, including medicine, dentistry, agriculture, veterinary science and law, to spend their first year at Townsville. A graduate school of tropical veterinary science was founded last year.

The situation of Townsville on the Coral Sea coast near the Great Barrier Reef is ideal for marine scientists. There will be opportunity for collaboration with the Australian Institute of Marine Science, which is to be set up at Townsville. The university is to have its own department of marine sciences, and has been allocated funds for a research vessel.

In the department of chemistry, where particular emphasis is placed on carbohydrates, research interests include the structure and metabolism of polysaccharides of tropical flora, and aspects of ruminant metabolism, as well as more fundamental chemical topics. Research interests in the department of physics are principally in radiophysics, wave propagation and low energy nuclear physics. The department is also responsible for the Tropical Research Unit in Atmosphere and Meteorological Physics.

In the department of engineering and geology, courses and research are oriented particularly towards industrial and mining developments in Queensland and other parts of Australia. Close liaison is encouraged with private industry and government agencies, which supply scientists who make specialist contributions to the teaching of the university.

As its graduate studies extend in scope, the James Cook University will increase its intake of students from South-East Asian, African and other overseas countries, especially in subjects of vital importance to tropical development, for example, veterinary science and engineering. It is one of the aims of this university to make its facilities available to other countries with tropical needs, as well as serving North Queensland and the rest of tropical Australia.

Parliament in Britain

Military Hovercraft

THE government has no intention of abandoning any projects concerned with military hovercraft development. This assurance was given by Lord Delacourt-Smith, Minister of State, Ministry of Technology, in reply to a question from the Earl of Kinnoull. He said that the BH7 model, which is now undergoing trials, has already been bought by the Ministry of Technology for evaluation by the Navy and that five models of the SRN 6, four of the SRN 5 and one of the SRN 3 are in service. The government is also discussing the possibility of assistance for further development both of existing and of planned craft with the British Hovercraft Corporation. (Oral answers, Lords, April 14.)

CS Gas

ALTHOUGH Mr Eric Moonman said that the United States produced 1,207,000 lb of CS gas in 1967, 4,251,000 lb in 1968, and 6,063,000 lb in 1969, under licence to the British government, he was told by Mr Ivor Richard that no money has been received arising from the licensing arrangements. (Written answers, April 16.)

Power Stations

MR ALAN WILLIAMS, joint parliamentary secretary, Ministry of Technology, said that on January 1 this year, the Central Electricity Generating Board had 46,035 MW total generating capacity. This consisted of 35,957 MW coal fired, 4,766 MW oil fired, and 3,138 MW nuclear, and the rest was made up from coal-gas fired stations and other sources. (Reply to a question from Mr Eric Ogden, written answers, April 16.)

Aerospace Industry

THE British aerospace industry was the subject of a wide-ranging debate in the House of Lords last week. Discussion touched upon almost every aspect of the industry, and was liberally sprinkled with pleas for Britain to become more involved in space research. The Earl of Bessborough, who opened the debate, said, for example, that no country which wishes to remain in the forefront of advanced technology can fail to be involved in space, and if the British government can go in with the United States in the development of the space shuttle, then it ought to do so. But that viewpoint did not entirely carry the day. In characteristic style, Lord Ritchie-Calder declared his opposition to manned spaceflight. He said that he has always deplored the Apollo mission for its waste of human ingenuity.

As far as the British aerospace industry is concerned, Lord Delacourt-Smith said that the size of the industry must reflect the amount of work which it manages to obtain, and that it is not the business of the government to keep it at any particular level. He also said that even though the future of the industry may seem to lie in international cooperation—especially with European countries—it is not government policy to pursue this course willy-nilly and rule out national projects automatically. The Minister of State concluded by saying that although the government may play a major part in the British aerospace industry, it is essentially for the industry to carry out its own long-term planning in the light of its own commercial judgment. (Debate, Lords, April 15.)

NEW WORLD

Short Commons for NSF (Part 2)

THE opening of the Senate's inquiry into the budget of the National Science Foundation on April 16 served principally to underline the seriousness of the foundation's plight, already apparent from its application for funds to the House of Representatives some weeks ago (see *Nature*, **225**, 785; 1970). The foundation now seems to be reckoning that it would take $50 million to support the research projects likely to be abandoned by the Department of Defense and more still to make up for the reductions of expenditure by other mission agencies of the US Government. According to Dr W. D. McElroy, director of the National Science Foundation, however, the $10 million in the foundation's budget originally set aside for abandoned projects will have also to be spent on the increasing demand for research money from young investigators.

If, however, the prospect ahead is bleak, the National Science Foundation seems to have found for itself a new champion in Senator Edward Kennedy, the chairman of the Special Subcommittee on the National Science Foundation set up by the Labor and Public Welfare Committee of the US Senate. For one thing, Senator Kennedy has already introduced into the Senate a bill that would increase the National Science Foundation's budget in the coming year by $50 million, although it is too soon to know how effective a rallying point that will be. He is also seeking to dramatize the foundation's plight by inviting to a public inquiry on April 23 the four former Scientific Advisers to Presidents of the United States—Dr James T. Killian, Dr George Kistiakowsky, Dr Jerome Wiesner and Dr Donald Hornig. According to Senator Kennedy, the intention is that the Scientific Advisers should "appraise the current needs of the NSF and the scientific community", and it is also expected that Dr L. DuBridge, the current holder of the office, will be represented at the same time. Whether this public demonstration will melt the heart of Congress only time will tell, but it may well suggest that Senator Kennedy will later this year inherit the reputation of Representative Emilio Q. Daddario as the chief defender of the NSF in Congress when, in November this year, Mr Daddario seeks election as Governor of Connecticut.

Introducing the inquiry last week, Senator Kennedy said that "science and scientists are facing both unprecedented criticism and unprecedented opportunity", with some critics saying that too much is being spent on defence and too little on the environment. But in his view, "our scientific community may soon be better able to serve our domestic needs than it has been for a generation", chiefly because of what seems to be willingness to "reorder priorities". In spite of the increase in the foundation's budget for 1971, Senator Kennedy said that "the Administration's authorization does not adequately take into account the overall research picture".

Dr Philip Handler, speaking as chairman of the National Science Board, had a gloomy tale to tell. He said that the National Science Foundation has not fulfilled the promise of the chief agency for the support of science in spite of its successes in the fields in which it has been able to operate. He complained that federal support for scientific research began to taper off in 1968 and has been virtually stagnant since. It was all very well to ask that science should be more concerned with projects bearing on social problems, and the Mansfield amendment (not necessarily to be repeated in 1971) was a sign of this, but it was wrong to "warp scientific endeavour by concentrating too much on immediate problems". He pointed to the way in which the National Institutes of Health were now reducing expenditure on organic chemistry, simply because of the narrower view now held of what constitutes "health related research", but thought it ironical that this kind of research "is the only source of new ideas for an industry worth $40,000 million a year". Given that there is no reason to think that science in the future will be less productive than in the past, he pleaded for an agency with the broadest possible licence for the support of scientific research. He said that he had been "deeply troubled" at the meeting last week of the Federation of American Societies for Experimental Biology in Atlantic City by the conflict between the eagerness of young scientists to help with social problems and their feeling that "if the Federal Government has lost interest, perhaps they have made a bad choice of a career".

Dr McElroy said that it is too soon to assess the full effect of the Mansfield amendment, but that the good projects left without funds are likely to be worth between $50 and $60 million a year. He said that the foundation had been surprised by the comparatively rapid growth of new applications from young investigators, up by 17 per cent at the beginning of April, and he explained that these applications would also be a charge on the foundation's spare cash. Within its planned budget, however, the foundation is going to be quite generous to the social sciences: institutional support will increase from $0·5 million in the current year to $4·5 million next year. The disparity between the foundation's ambition and what it has to spend is perhaps most clearly illustrated by Dr McElroy's guess, in reply to prodding from the chairman of the subcommittee, that it would be easy to spend $800 million, more than half as much again, in 1972.

At the inquiry last week, there was a good deal of anxiety about the way in which the foundation has been asked to strike out from its budget the support of graduate students under its traineeship programme; although plans for this September will not be affected, Dr McElroy said, "I understand that the Administration is looking into the possibility of financing graduate students by means of loans". There is some optimism about the prospect of attracting laboratories such as Oak Ridge, Brookhaven and the Argonne Laboratory into some of the foundation's plans for multi-disciplinary research. Everybody's head was shaken in sorrow at the curtailment of the foundation's college improvement programme—in the current year, the foundation has been able to spend only $4·4 million on 575 proposals when it calculates that something like $10 million worth of valuable projects were included in the 1,600 proposals.

More Delay at Arecibo

One of the disappointments of Dr McElroy's statement to the Kennedy subcommittee was the news that the project to resurface the radio telescope at Arecibo in Puerto Rico has been postponed pending an engineering design study of the problems involved in mounting an exceedingly thin skin of perforated aluminium on the wire framework now being used. The original proposal was for an aluminium skin 0·008 inch thick perforated by square holes an eighth of an inch across to let wind and water through. The cost of the modifications, at the top of the list of projects suggested two years ago by the committee under Professor R. H. Dicke, was $3·8 million. By all accounts, the National Science Foundation was dissuaded a year ago from applying for the money. Dr McElroy said last week that a study of the proposal has now "raised a number of questions concerning problems relating to the installation of the proposed surface".

The feasibility of the project now depends on engineering design studies, and a new estimate of costs will be worked out on that basis. Dr McElroy said that he will go back to the appropriate Congressional committee with the new estimate as soon as this is available.

Dr Robert Fleischer, head of the astronomy section at the National Science Foundation, explained that the inadequacy of the original estimate of cost had come to light during a detailed examination of the proposal. He said that it now looks as if the original estimate might be low and that the foundation was anxious not to seem as if it had gone to Congress with an unrealistically low estimate in the hope of securing a degree of commitment that would not otherwise have been forthcoming. There is no doubt that the engineering problems are considerable. The intention is to resurface the dish, which is 1,000 feet across, so that it can operate at a wavelength of 6 cm, which means that the surface has to be geometrically accurate to within about 3 mm. It now looks as if a heavier surface than that originally planned will be necessary. One of the difficult points that will arise towards the end of the proposed engineering study, possibly six months from now, is that the cost of resurfacing Arecibo may be so great that a reordering of radio astronomers' priorities for new instruments will seem appropriate.

The second proposal in Professor Dicke's list, that for a 400 foot steerable dish enclosed in a radome and operated by a consortium of universities in the north-east, has moved a little forward, or at least sideways. Apparently with the tacit consent of the National Science Foundation, the natural sponsor, the Smithsonian Astrophysical Observatory, one of the consortium of would-be builders, is applying separately to Congress for funds to carry out a thorough design of the telescope. Although success is unlikely, this development does illustrate the despair which radio astronomers share.

TECHNOLOGY

Mr Wedgwood Benn Swings By

Mr Anthony Wedgwood Benn, who began his week in Washington on April 15 with a combined cocktail party and lantern slide lecture, left for Los Angeles after raising an astonishing number of questions with departments of the US Government. Little on the agenda has turned itself into formal agreements, and Mr Benn was careful to explain that that had not been his intention. On the question of whether the United Kingdom, with or without its European partners in ELDO, should collaborate with the United States in future space projects such as the space shuttle that NASA wishes to build, Mr Benn said, for example, that he would want to discuss several possibilities with the other members of the "lunch club" of European ministers inclined to take some interest. Mr Benn seems to think of Europe more as a customer of the American space programme than as a participant in it. No doubt the next European Space Conference in Brussels in July will help to clarify the position.

Mr Benn seems to have raised some interesting issues in his talks with the Department of State, chiefly on matters such as international technological collaboration on questions such as standardization. He has clearly been at pains to emphasize the potential importance to Britain of the various schemes for technical collaboration now being fostered by the Aigrain committee within the EEC, and of the possibility of British membership of the community. One of his views is that the time has come for governments such as the British Government to "open diplomatic relations with the international companies", and he said that he and American counterparts had agreed to exchange experiences. The environment seems to have featured in Mr Benn's talks both with Dr L. DuBridge and Mr Russell Train, the chairman of the Environmental Council. Although Mr Benn says that "my heart is in the environmental business", a part of his objective seems to have been to convince American officials that there must be coordinated international progress in the development of standards if some of the potentially awkward consequences of the improvement of the environment are to be avoided. One illustration of the difficulty of which British motor-car manufacturers are still vividly aware is the way in which the rapid introduction of stringent safety standards for American automobiles three years ago cut into British exports of some models. Last week, Mr Benn pointed out that the United States could not "ban the internal combustion engine by Thursday week" without sending all kinds of economic ripples about the world.

Among the other subjects on this crowded agenda was the Harrier aircraft, now being evaluated by the US Air Force, and the European consortium's plan for the development of the gas centrifuge, which Mr Benn talked over with the Atomic Energy Commission. Transportation seems to have played an important part in his discussions, both where the supersonic transport is concerned and with matters such as fast ground transport and automobile engines, where it has apparently been agreed that the two governments will keep in touch with each other.

Indeed, the most difficult meeting that Mr Benn expected to have in Washington during his current visit was with Mr John Volpe, the Secretary for Transportation. The objective was to obtain convincing reassurances that the United States was not going to ban supersonic overflights to the disadvantage of Concorde and then relax the rules later when American supersonic transport comes along. The Concorde is considered to have a comfortable five year lead over the American project at present.

At least Mr Volpe's official statement last week that a ban on supersonic overflights by civil aircraft is in view brought some clarification. In very carefully chosen language Mr Volpe gave notice of a proposed ruling which would ban all supersonic flights over the United States where it can be proved that the sonic boom will reach the ground. Exemption will be given to operators who can demonstrate that no sonic boom is generated by a supersonic flight. Perhaps this language need not be taken too seriously. It seems that Mr Volpe was adopting a procedure for alerting interested parties—such as Mr Benn—to future regulations before they finally crystallize. Comment on the ruling is invited up to June 15, and Mr Benn seems to have reacted swiftly. The day after the announcement he offered to provide all the data of Concorde test flights over land to allay American fears on Concorde's "noise pollution" characteristics. Ironically, the Ministry of Technology spends more on research into noise than on any other form of pollution. Of course, this is nothing like as much as it spends on Concorde.

New GRAS List

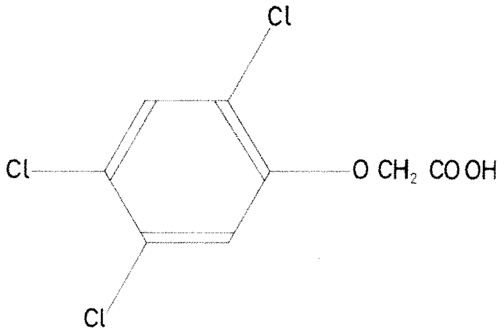
The day after the appearance of the latest Nader report, but not on that account alone, the Food and Drug Administration asked that food manufacturers in the United States using food additives licensed in the palmy days before 1958 should reapply to have these included in the list of materials generally recognized as safe (GRAS). An official of the FDA explained that when the present formal procedures for including materials on the GRAS list were introduced in 1958, a large number of compounds used with food additives had been approved on the basis of advice of variable quality and in comparatively informal ways. What has happened now is that food manufacturers have been asked to reapply for the inclusion in the GRAS list of food additives originally approved before 1958. The FDA has undertaken to give some kind of an answer within 30 days. By all accounts, it does not know how many compounds will be affected—it may be anything between 1,000 and 10,000. Much will depend on the extent to which previous permissions are subsumed in later more formal approvals.

Another Herbicide on the Blacklist

Herbicidal chickens from Vietnam came home almost literally to roost last week when Dr M. Jacqueline Verrett of the Food and Drug Administration showed off to the chairman of a congressional committee two trays of deformed chicks born of eggs dosed with the controversial herbicide 2,4,5-T. She followed the surgeon-general of the United States, Dr Jesse L. Steinfield, who told the Subcommittee on Energy, Natural Resources and the Environment of the Senate Committee on Commerce that the secretaries of Agriculture, the Interior and Health, Education and Welfare had decided to restrict the use of 2,4,5-T in circumstances when foodstuffs for human consumption might be contaminated. With his tongue ostentatiously in his cheek, Dr Julius E. Johnson, director of research at the Dow Chemical Company, told the subcommittee that he did not think this decision was warranted if the issue was simply a matter of public health, although "in the climate of today, I think it was a wise decision". The inquiry did, however, throw light on several apparent conflicts in previous reports about the hazards of 2,4,5-T by showing that many of the mutagenic and teratogenic effects attributed to 2,4,5-T are really caused by a much more toxic impurity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This material and its congeners are likely to attract a good deal of attention in the months ahead.

Dr Steinfield said on April 15 that the evidence on which the three departments had decided to ban 2,4,5-T had become available only at the previous weekend, which goes some way to explain why officials of the United States Department of Agriculture had taken a more complacent line about the herbicide in their evidence to the subcommittee only a week before. The immediate effect of the decision is to suspend current permissions (or registrations) for the use of liquid preparations of 2,4,5-T domestically and on lakes, ponds and ditches. At the same time, the United States Government has revoked permissions to use non-liquid preparations of the herbicide domestically and on crops of all kinds. Preparations in the first category will remain in limbo unless further research allows the restriction to be relaxed. It will still be possible legally to use 2,4,5-T for the control of weeds on pasture land, footpaths and forest ground, which means that people who are sensitive to poison ivy and poison oak, two of the natural scourges of North America, can still hope for some relief by the use of the weed-killer which is most effective against them.

Dr Steinfield's announcement is a victory, even if somewhat muted, for groups of scientists who have campaigned against 2,4,5-T on two separate counts during the past six years. The herbicide has been widely used in Vietnam as a defoliant, and the American Academy for the Advancement of Science has done stalwart if somewhat intermittent work to draw attention to the long-term ecological hazards of the practice. (A committee under Professor M. Mendron of MIT plans to have a report on the subject by the end of 1970.) More recently, there has been concern about the potential teratogenic effect of 2,4,5-T, especially in an article in the *New Yorker* by Mr Thomas Whiteside last December. This is the point which has now been conceded by the United States Government, although Dr Steinfield also announced last week that the Department of Defense had also asked those responsible for defoliation in Vietnam to pay



2,4,5-Trichlorophenoxyacetic acid

heed to the findings which have now come to light.

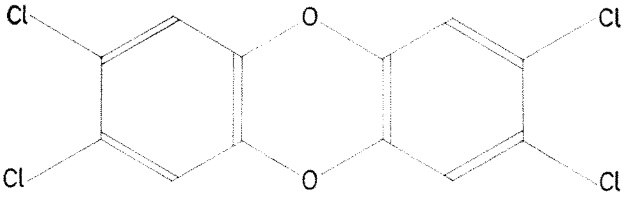
The inquiry last week showed that impurities in 2,4,5-T must play an important part in any discussion of its toxicity, and the way in which this has been worked out promises to become a classic in the history of chemical detective work. The Dow Company and Dr Verrett seem to take much of the credit. The herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is a synthetic auxin which kills weeds systemically. Like the similar material 2,4-dichlorophenoxyacetic acid (2,4-D) it has featured in the rapid growth of herbicide applications in the United States in the past decade or so. Although there have been reports, necessarily hard to confirm, that the use of these materials in Vietnam has increased the incidence of congenital malformations among people, the first objective evidence of a teratogenic effect of commercially available 2,4,5-T seems to have been provided by a lengthy search for teratogenic effects in pesticides (forty-eight materials altogether) carried out between 1965 and 1968 by the Bionetics Research Laboratories, under contract with the National Cancer Institute. More than a year after the Bionetics studies were completed, on October 29, 1969, Dr L. DuBridge in his capacity as executive officer of the Environmental Quality Council announced that the use of 2,4,5-T by departments of the federal government would be restricted, and that the use of the material in agriculture would be curtailed unless safe limits could be established by the beginning of 1970. One of the muddling features of this case to emerge at the inquiry last week was that the influential Mrak Commission on Pesticides (which counted herbicides in its field of interest) was not told of the results of the Bionetics study until Dr DuBridge's announcement. Another is that the Department of Agriculture seemed at first studiously to ignore what Dr DuBridge had to say, which is why last week's developments were something of a vindication of his reputation.

According to Dr Julius Johnson of the Dow Chemical Company, one of several manufacturers of 2,4,5-T, his company responded to the announcement last October by making a search for impurities in the product. The herbicide is manufactured from 1,2,4,5-tetrachlorobenzene by hydrolysis (to form the sodium salt of 2,4,5-trichlorophenol) which is then treated with the sodium salt of monochloroacetic acid. Dr Johnson said that "since 1950 we have been keenly aware" that highly toxic compounds are formed from 2,4,5-trichlorophenol at high temperatures, and in 1965 the chief offender was identified as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This happens to be not merely one of the agents responsible for sporadic outbreaks of an occupational disease most recognizable as a severe acne among workers at chemical plants, but also as one of the contaminants in chicken food fortified with fatty acids now known to be responsible for the "chicken oedema disease" which has plagued poultry farmers for close on two decades. According to Dr Johnson, the Dow plant was modified in 1965 so that the amounts of dioxin in 2,4,5-T were less than 0·5 parts per million. The inference is that materials in circulation before 1965 included larger proportions of the dioxin, and the sample used in the Bionetics study (manufactured by the Diamond Alkali Company) has now been shown by gas–liquid chromatography to contain 27 ± 8 parts per million of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Although information about the manufacturing hazards seems to have been shared within the chemical industry, Dr Johnson's account last week suggests that the possibility that dioxin in herbicide could cause teratogenic effects in mammals was taken seriously only in late October last year, when Dr DuBridge made his announcement and the results of the Bionetics study were communicated to the Mrak Commission, of which Dr Johnson was a member.

In the past six months, studies of 2,4,5-T and its chief dioxin contaminant have been carried out by the Dow Company, the National Institute of Environmental Health Sciences and the Food and Drug Administration, chiefly in the search for teratological effects. The surgeon-general said last week that the decision to restrict the use of 2,4,5-T had been based on the finding at the National Institute for Environmental Health Sciences that "nearly pure 2,4,5-T was reported to cause birth defects when injected at high doses into experimental pregnant mice but not in rats". According to Professor Samuel S. Epstein of the Children's Cancer Research Foundation, Boston, the chairman of the Mrak Commission's panel on teratology, who also appeared at the inquiry last week, these studies (in which pregnant mice were dosed with 2,4,5-T) indicated cleft palates "at dose levels of 150 mg/kg and scattered abnormalities at 100 mg/kg; the cleft palate incidence in control mice was essentially zero".

The Bionetics study in which the teratological effects of impure 2,4,5-T were first recognized was more alarming—in one series of experiments, for example, the oral administration of 113 mg per kg to AK mice on days 6 to 15 of pregnancy produced 49 per cent of deformed foetuses, among which cleft palate was conspicuous. The same study, with small numbers of animals, showed that Sprague–Dawley rats are also sensitive. These results are no longer a sure guide to the behaviour of 2,4,5-T because of the contamination of the sample used with dioxin.

The studies carried out by the Food and Drug Administration and described last week by Dr Verrett



2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

have been made with recrystallized 2,4,5-T and with samples of 2,3,6,7-tetrachlorodibenzo-*p*-dioxin. The technique of injecting these materials into fertile hen's eggs, which has been used in screening materials suspected of causing chicken oedema disease, produced the collection of malformed chicks which Dr Verrett exhibited last week and provided the committee chairman, Senator Phillip Hart, with a splendid opportunity to hold normal and abnormal chicks in front of the television cameras, but both the surgeon-general and Dr Johnson of Dow argued that chick embryo studies are not reliable guides to the potential teratogenic effects of chemicals in other species. Dr Verrett's purpose seems to have been to compare the potency of the various materials used, and from her statement it would seem that 50 micrograms of the new 2,4,5-T manufactured by Dow produces chicken oedema symptoms and structural abnormalities with the same frequency as 6·25 micrograms of the sample used in the Bionetics study. Recrystallized herbicide also produced abnormalities in chicken embryos, but only at doses five times as great. On this scale, there seems no doubt of the greater potency of the tetrachlorodioxin—one nanogram of this material seems to have produced disturbed embryos with noticeable frequency.

Dr Verrett also described last week studies with pregnant hamsters. Manufactured 2,4,5-T with an estimated 0·5 p.p.m. of tetrachlorodioxin killed 80 per cent of the foetuses at a dose of 100 mg/kg, but the three-times recrystallized sample produced 55 per cent of foetal deaths at the same dose and three out of the thirty-eight pups born alive turned out to be malformed. According to Dr Verrett, with a preparation that was still more carefully purified, early foetal deaths worked out at 70 per cent, but there were no "gross terata" at 100 mg/kg. The dioxin turns out to be roughly 1,000 times more effective—9·1 micrograms per kilogram produced 98 per cent of foetal deaths.

Dr Verrett's conclusion was that both 2,4,5-T and 2,4-D taken from production runs are teratogenic, and that even the purified sample, 2,4,5-T, is suspect, "making it impossible at this time to exonerate it of teratogenic and other adverse effects on the embryos that may have some health significance".

One of the disconcerting features of the results made public last week is that the three independent groups of investigators have used different test animals, which means that direct comparisons are complicated. The investigations carried out by the Dow Company have been with Sprague-Dawley rats, apparently by agreement with the Food and Drug Administration. 2,4,5-T from the Dow production line, containing an estimated 0·5 p.p.m. of tetrachlorodioxin, seems to have produced no embryo losses at doses up to 24 mg/kg, at which level, however, 29 out of 103 foetuses were deficient in sternebral ossification (compared with four foetuses in the control group of the same size). A separate Dow study has shown that the tetrachlorodioxin contaminant will stimulate the resorption of foetuses when fed to rats between the sixth and fifteenth days of pregnancy at 2 micrograms a day or more, that gastro-intestinal haemorrhage is found between 0·125 micrograms a day and 8·0 micrograms a day, and that there is a reduction of foetal weight within this range. Professor Epstein pointed out last week, however, that these results raise new issues, for cystic kidneys were rare among the congenital abnormalities produced in the most recent Dow studies, but common—and presumably the results of dioxin contaminant—in the Bionetics study. "This discrepancy may reflect synergistic interaction between dioxin and 2,4,5-T."

A somewhat breathless account of the latest Dow experiments by Dr Johnson suggests, among other things, that this may be so. To begin with, it was established that a dose of 100 mg/kg/day of 2,4,5-T would kill 75 per cent of pregnant rats, but that the animals could tolerate half that amount with considerable physiological stress, including the resorption of foetuses. Results which, last week, "were only available yesterday" show that the addition of microgram quantities of the tetrachlorodioxin to this dose produces significant teratological effects only at the level of 6 micrograms/kg/day (in experiments in which pregnant rats were fed the materials between the sixth to the fifteenth days of pregnancy). Eight per cent of 155 rats born after such a regimen showed signs of gastro-intestinal haemorrhage. With the addition of 0·5 micrograms/kg/day and twice that amount respectively to the base level of 50 mg/kg/day of 2,4,5-T, four and five out of fifteen newborn rats were found to have cleft palates.

This is the point at which Dr Johnson entered his most vigorous defence of 2,4,5-T. He started from the surveys of the Food and Drug Administration which have sought evidence of the herbicide in actual samples of food, and which have revealed only trace quantities —less than 0·1 part per million—in 25 out of 5,300 food samples, with one sample containing 0·19 p.p.m. and another 0·29 p.p.m. On the assumption that all food contains 0·2 p.p.m. of 2,4,5-T and that this is contaminated with 1 p.p.m. of tetrachlorodioxin, a person would accumulate a dose of 11 micrograms in 100 years. This, according to Dr Johnson, is a "600-fold safety factor compared with what we estimate to be the embryo-toxic effects in rats", with the proviso that the whole amount must be delivered during the appropriate stages of pregnancy. This calculation was the basis for the air of puzzled resignation with which Dr Johnson greeted the restriction on the use of 2,4,5-T—but Dow reserves the right to appeal against the new regulations when these are published.

By the end of the inquiry, however, the prospect of contamination by other kinds of chemicals had captured the attention of the subcommittee. Dr Verrett was the first to point out that no account had been taken of the still unknown teratological effects of the large numbers of isomers of tetrachlorodibenzo-*p*-dioxin which might also be present in manufactured herbicide. She went on to chill the subcommittee with the news that the way in which these materials can be produced by the pyrolysis of substituted chlorophenols means that there are potential hazards in the burning of great variety of materials to which various chlorophenols are added during manufacture—paper, leather and the like. Professor Epstein gave a more deliberate recitation of the same case—he argued forcefully for more information about the formation of dioxins from all kinds of chlorophenols and more information about the teratogenicity of the dioxins. "Available data . . . indicate an urgent need for restriction of human exposure to dioxins. Similar restrictions should extend to polychlorophenols, polychlorophenolic containing formulations and their combustion products."

NEWS AND VIEWS

Squeezing some More from the Earth

It might have been thought that once seismology had presented a clear picture of the elastic properties of the Earth it could disappear gracefully from the scene. The trouble, or to the insider the virtue, is that the more carefully the Earth is studied the less it is possible to fit its elastic properties into any simple spherically symmetric model. The structural difference between the upper mantle under continents and under oceans is believed to extend to a depth of at least 600 km. Substantial slices of the mantle in which deep earthquakes occur (for example, round the Pacific) are known to be out of thermal equilibrium with their surroundings. The techniques for determining earthquake mechanisms have been improved beyond all recognition in the past three or four years. And commanding increasing support is the earthquake prediction programme, which is quietly being renamed the earthquake control programme. So the future still holds lots of prizes for the seismologist.

New problems are very often best attacked with new tools and in the past two years there has been a spate of instrumental developments. Coverage of the world with short period (1 s) instruments is now reasonably good. These instruments are of particular use in location of events, and work with magnifications up to a million. There are several "holes" in global coverage—the Pacific Ocean, the Arctic, Africa—but stations on Pacific Islands, in Northern Canada and Siberia and in Kenya, Ethiopia, Egypt and South Africa help to plug the gaps. The longer period instruments (10 s up to thousands of seconds) are less thick on the ground and are particularly inadequate for periods greater than 40 s. Such seismometers are of use in studies of earthquake mechanism, distribution of strain patterns before and after earthquakes and study of the deeper structure of the Earth by long-period surface waves and free vibrations of the Earth. It goes without saying that they are of considerable value in nuclear test detection. The number of such seismometers in use, however, is now increasing rapidly. Particularly noteworthy are the novel designs that are being used.

At least four new types of laser seismometer have been designed in the past two or three years. These are strain measuring devices which work as interferometers with automatic fringe detection. Strains as small as 1 in 10″ may be measured and laser seismometers with a broadband response are extremely versatile seismometers. Because the optical paths are usually *in vacuo*, environmental control need not be stringent. Although two or three work in disused railway tunnels, one (800 m long) sits on the ground basking in the Californian sunshine.

Last year some impressive new results were demonstrated by Molnar *et al.* (*Nature*, **224**, 1268; 1969) using careful environmental control of a conventional long period seismometer and improved amplification devices. Undoubtedly these new Lamont seismometers will open up fields of research in a part of the spectrum so far very inadequately treated.

In this issue (page 343), Block *et al.* describe some of the results recently obtained with the new accelerometer developed at Scripps during the past three or four years. This instrument is interesting in that it was designed by physicists to see how good an accelerometer could be made, rather than by geophysicists with particular problems in mind. The result is quite remarkable. Block *et al.* report very clear excitation of the free oscillations of the Earth from a magnitude 6·5 earthquake. There are generally at least twenty earthquakes of this magnitude or greater every year. To put this achievement in perspective we may note that free oscillation observations had only previously been possible from earthquakes with magnitudes greater than 7·7, and that only two earthquakes (Chile, 1960; Alaska, 1964) have received widespread attention. It is reported that, since the New Hebrides earthquake, several other earthquakes have allowed the measurement of free oscillations.

It seems that a new era of instrumental development and increased seismological knowledge is opening. The subject is clearly going to be as lively in the next decade as it was in the last.

COMETS

Another Hydrogen Cloud

by our Astronomy Correspondent

A second example of a cloud of hydrogen surrounding the head of a comet was reported last week by NASA. Observations from one of the Orbiting Geophysical Observatories show that comet Bennett which first appeared over Australia in January is immersed in a cloud of hydrogen about eight million miles across—ten times the diameter of the Sun.

This follows the news of a hydrogen cloud surrounding the comet Tago-Sato-Kosaka, announced by NASA earlier this year from data obtained with the Orbiting Astronomical Observatory, OAO 2 (see *Nature*, **225**, 413; 1970). The size of the cloud around Tago-Sato-Kosaka was said to be comparable with the size of the Sun.

Although the hydrogen clouds are unexpected, they are fairly simple to account for on the current model for cometary nuclei. As the comet approaches the Sun some of the gases which are contained in the mass of mineral particles and frozen gases which make up the nucleus would be expected to boil off. The problem is that the coma of gas surrounding the nucleus, which is recognized to be the result of this process, is tiny by comparison with the hydrogen clouds now observed.

The reason why the hydrogen clouds have not been observed before is that the ultraviolet radiation which they emit is at too short a wavelength to penetrate the atmosphere. Both clouds were discovered with detectors sensitive to the Lyman α line of hydrogen at 1216 Å. Following the discovery of the cloud around Tago-Sato-Kosaka, the Lyman α detector on OGO 5 was pointed at comet Bennett on April 1 and 2 for two scans across the cloud. The detector in OGO 5 was developed by Professor J. E. Blamont's team at the University of Paris, and a preliminary map of the hydrogen distribution shows the cloud slightly elongated away from the Sun, as might be expected.

Comet Bennett was at its maximum brightness of 1·5 stellar magnitudes between March 21 and 25. According to NASA, there are also plans to observe the hydrogen cloud from OAO 2 and possibly using Mariners 6 and 7—the spacecraft equipped with ultraviolet detectors that last summer made close approaches to Mars.

LIGHT

Polarized Beams Divided

CIRCULARLY polarized light will be reflected in a different way from a surface depending on whether the polarization is to the left or to the right, according to C. Imbert of the Orsay Institute of Physics (*Phys. Lett.*, **31**, 337; 1970). In an experiment involving two different shapes of prism, he has shown that when two beams of light circularly polarized in opposite senses are totally reflected from a surface a separation appears between the beams which, when amplified, can be directly observed.

Although this strange result has been predicted from solutions of Maxwell's equations of electromagnetism, this seems to be the first time that the effect—known as the photon's translational inertial spin effect—has been confirmed experimentally. What it means, effectively, is that the momentum and velocity of photons in a circularly polarized beam of light are not collinear, or at least not collinear in the conditions of this experiment.

A beam of laser light was split into two circularly polarized parts by inserting a quarter wave plate followed by a half wave plate covering half the beam area. The light then fell on to a prism with one totally reflecting surface, and the expected divergence between the beams was multiplied by a series of internal reflexions inside the prism. Two types of prism were used, one giving twenty reflexions and the other thirty.

A rectilinear object with a clear edge was interposed between the half wave plate and the prism. The separation between the beams was then visible on the final image as a shift between the two halves of the edge. Imbert found that the size of this shift agreed with predictions to within the fairly generous experimental error. He points out that for circularly polarized light the momentum and velocity of a beam are not necessarily non-collinear, but that for total reflexion from a surface this separation seems now to be established.

THIN FILMS

On the Surface

from a Correspondent

THE newly formed Institute of Physics Thin Films and Surfaces Group held its first conference at the University of Reading from April 6 to 8. The theme was electronic properties of surfaces and thin films. Almost half of the contributions concerned aspects of amorphous thin films, which were introduced by Professor Sir Nevill Mott (University of Cambridge), who reviewed theories developed from a model of random depth potential wells. He pointed out that the plot of the logarithm of the observed conductivity against reciprocal temperature could be explained in terms of conduction in non-localized states at higher temperatures, by hopping between localized states at the bottom of the band for medium temperatures and by phonon assisted tunnelling by electrons at the Fermi level at low temperatures. Further experiments, for example, comparing thermo-power with conductivity and deliberately introducing acceptors by ion implantation, could provide more evidence about this model. In this context Drs J. G. Perkins and P. T. Stroud (Atomic Weapons Research Establishment, Aldermaston) showed that their technique for "knocking-on" atoms from a metal top electrode into silicon oxide films using 50–80 keV argon ions gives extremely reproducible results.

Dr R. M. Hill (Chelsea College of Science and Technology) showed how the conductivity and its temperature dependence could be explained by considering just one conduction mechanism, involving tunnelling and field emission from Poole–Frenkel defect centres, over the whole temperature range.

Professor A. K. Jonscher (Chelsea College of Science and Technology) presented an impressive collection of experimental data, obtained from amorphous SiO using photocurrent measurements, showing great reproducibility for various different contact metals and polarities of the applied voltage.

Clearly there are still theoretical problems, from the discrepancies between the various ideas presented, but at least it seems that the experimental results are consistent enough to apply theory.

Moving from amorphous to polycrystalline films, Professor J. C. Anderson (Imperial College, London) showed how the Fuchs–Sondheimer theory of surface scattering could be applied to semiconductors and extended to include the effect of surface band bending. He also showed how scattering at grain boundaries can have a large influence on the transport properties of polycrystalline films. Dr J. H. Fisher (Imperial College, London) showed how a similar theory could explain the Hall effect data from thin InSb films. For epitaxial semiconductors Dr M. R. Jordan (Plessey Co., Caswell) showed that PbSnTe films gave better than the best bulk results so far reported for mobility and that the films would be useful as infrared detectors in the 10 μm region.

When attention turned to semiconductor surfaces, Dr B. H. Blott (University of Southampton) reviewed theoretical and experimental work involving low energy electron diffraction and back scattering on the structure of surfaces. He pointed out that it is easier theoretically if the inelastically scattered electrons are used.

Dr V. Heine (University of Cambridge) reviewed some of the theoretical work on surface states which predicts the intrinsic states on both diamond and zincblende types of semiconductor. For an oxide coated surface the surface states could be caused by charges in the dielectric. Dr M. Pepper (Plessey Co., Caswell) showed that there is a correlation between the slow states and fast states for the Si–SiO_2 system. From theory Dr A. A. Cottey (University of East Anglia) predicted that there may be a size effect associated with a little known state of an electron at the band edge in a crystal.

Continuing on size effects, Dr R. J. Cruickshank (University of Aston) discussed results which showed that the galvanomagnetic properties of Bi were an oscillatory function of thickness. This was explained in terms of standing Bragg waves in the crystallites.

Professor H. Mayer (Clausthal) reported experiments using the photo-electric effect to excite surface and volume plasmons in alkali halide films. He pointed out that the use of thin films enabled the surface plasmon frequency to be measured. Dr R. H. Ritchie (Oak Ridge National Laboratory) also considered plasmons when he reported some novel ways for studying the surface structure using coupling between surface modes.

METEOROLOGY

No Clouds for Nimbus

THE most detailed mapping ever of the temperature of the atmosphere has just been sent back from a British experiment on the American Nimbus 4 satellite, Dr B. J. Mason, director-general of the Meteorological Office, told a meeting of the Royal Meteorological Society in London last week. In the six days since Nimbus 4 was launched on April 8 more than 30,000 temperature profiles were accumulated from infrared spectra of carbon dioxide in the atmosphere, providing valuable information for future long range weather forecasting. With the satellite sweeping out thirteen orbits of the Earth each day it is hoped to compile an accurate chart of the atmosphere up to an altitude of more than 50 km.

Encouraged by the results of the Nimbus programme, in which two satellites still remain to be launched, Mason believes that satellite experiments may revolutionize the study of medium scale air motions, which affect areas up to three or four hundred miles across. Large scale air movements across whole continents can usually be well simulated in numerical models, he explained, but motions on a smaller scale are poorly understood and have to be represented on a statistical basis. The physics of medium scale motions are still too unclear to know just how inherently predictable the atmosphere is.

A large tropical observing experiment is to be mounted in 1974 to study how heat is transferred from the tropics to other regions and to investigate the air–sea interaction. Mason hoped that Britain would participate in this research, and foresaw a further two or three similar experiments being set up before the end of the century.

The most significant advance in forecasting in the next ten years is likely to occur in five to seven day predictions. The demand for forecasts is growing by 20 per cent each year and the gas and electricity authorities are now asking for hourly temperatures predicted up to three days in advance. This is not yet possible, but it is hoped to introduce rainfall predictions for up to two days ahead on a 40 km grid across Europe as soon as a new computer is introduced in 1971.

Thirty day forecasts are improving all the time. Mason pointed out that there was only one total failure in Britain last year out of twenty-four predictions, compared with five for 1968. He saw this as the area of forecasting most likely to benefit from an improved understanding of the atmosphere during the next thirty years.

The best chance of improving long term forecasts lies in numerical models, Mason said. At present the limiting factor is the speed of computer calculations, but tardy dissemination of data is also a serious handicap. A chart which takes twenty seconds to produce on a computer can take up to twenty minutes to transmit elsewhere. Digital techniques are now being deployed in the United States to reduce this time to less than a minute, and for a station such as that at Bracknell producing 400 charts a day this is clearly an important bottleneck to loosen.

ANTARCTICA

Glacial History

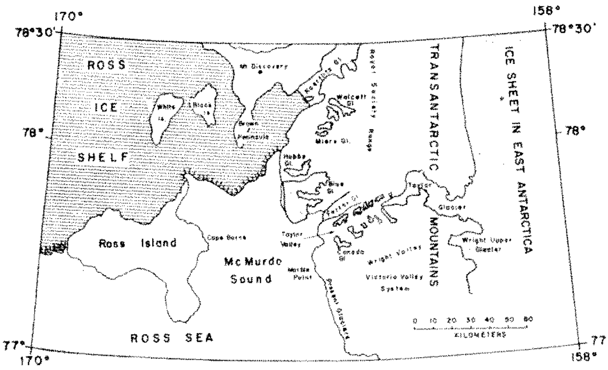
from our Geomagnetism Correspondent

THE most important questions to be asked about the Antarctic Ice Sheet are the obvious ones: What is its history ? When and why did it form ? Has it existed continuously since its origin or has it ever partly or completely disappeared ? Has it undergone changes in area and volume, and if so, were these changes contemporaneous with worldwide glaciations ? And from the past, what can we infer of the future ? Will the future behaviour of the ice sheet involve large surges, widespread melting, stability, or continuation of the present growth ?

These are some of the questions which Denton *et al.* (*Antarctic J.*, **5**, 15; 1970) set out to answer, at least partially, by an investigation of the ice-free zones in the region of McMurdo Sound, Victoria Land. This is a unique area for a study of glacial history in that it is a melting pot for no less than three major glacial systems (see map). First, the East Antarctic ice sheet is dammed west of the Transantarctic Mountains, although two small tongues of the ice sheet, the Taylor and Wright Glaciers, spill over into the western ends of glacially carved valleys. Second, in the east the Ross Ice Shelf floats on the surface of the Ross Sea. And finally, between the two glacial systems, occur independent alpine glaciers.

Denton and his colleagues have tried to compile a chronology for the three-glacier system which is, as far as possible, absolute, and they have been fairly successful. The various advances and recessions of the ice were, of course, deduced from a systematic examination of the glacial erosion features through the McMurdo Sound region. The dating of these changes was more difficult, because it depended much more on luck. Thus in some cases it was necessary to set glaciation limits in time by the potassium–argon dating of lavas and volcanic cones erupted between two glacial periods. In other cases, carbon-14 ages on shells in marine beds, freshwater algae in moraines or mummified seals resting on ice-free surfaces were used likewise.

The principal conclusion to emerge was that the huge ice sheet in East Antarctica had attained a "full-bodied stage" at least 4 million years ago. The build-up of ice and the corresponding 55 m fall in sea level must



therefore have occurred during or before the Pliocene. Other evidence has shown that West Antarctica was largely covered by an ice sheet by the late Miocene; and since the conditions necessary for this almost certainly prevailed in the east, too, it seems likely that the East Antarctica glacier had also been established by that time. This would presumably explain why eustatic sea level began a rapid decline during the Miocene. On the other hand, Tertiary sea level data give no indication of polar ice sheets before the Miocene. But this is not conclusive evidence; and the question of whether large glaciers existed in Antarctica before the Miocene remains open.

During the past 4 million years the Taylor–Wright glacier system has fluctuated in level several times, although the changes bore no relation to world-wide glaciations. At the moment the level is rising; and the ice is at its maximum height since before the Wisconsin glaciation as defined elsewhere in the world. Four Ross Sea glaciations can be recognized, all occurring within the past 1·2 million years. The recession of the most recent of these coincided with the rapid rise in sea level during the Late Wisconsin, but it is unlikely that this apparent correlation was climatically controlled because alpine glaciers in the area diminished in size during Ross Sea glacial advances. Further, the major Ross Sea glaciations were not synchronous with changes in level of the East Antarctic ice sheet. It is becoming clear that the three glacial systems in the McMurdo Sound area have fluctuated completely independently of each other.

CARBON

Glassy and Fibrous

from our Materials Science Correspondent

For some years, and especially since the Parliamentary Select Committee's report last year, attention in Britain has been focused on carbon fibres made by the British process developed at the Royal Aircraft Establishment. Much anguish has been aroused in political, engineering and industrial circles by uncertainties as to the proper manufacturing scale, price and applications of these fibres. The uncertainties are not eased by the fact, not widely recognized, that there are competing carbon fibres with an inferior technical specification but also a lower price.

Kawamura and Jenkins, who work at University College, Swansea, have described recently (*J. Materials Sci.*, **5**, 262; 1970) a new glassy carbon fibre made by pyrolysis of hexamine-tetramine dissolved in phenol, extruded to form threads. The extrusion and carbonization stages seem to be very simple and involve temperatures no higher than 1,000° C. A maximum tensile strength of 2 GNm^{-2} (3×10^3 pounds/inch^2) and Young's modulus of 70 GNm^{-2} (10×10^6 pounds/inch^2) for fibres 5 μm in diameter are reported. The raw materials can be derived from coal or oil tar fractions and the method has been patented by the National Coal Board.

There has been intensive development of a somewhat similar fibre in Japan, by the Kureda Chemical Industry Co. Ltd, based on unspecified "hydrocarbon pitch", particularly during the past year. This, and related work on other forms of carbon fibre, have been described in a recent survey by A. J. McLaren (*Design Engineering*, 109; February 1970). The carbonization temperature in the Japanese process is again about 1,000° C. The mechanical properties quoted are similar to those of the Swansea fibre, though the strength seems to be somewhat lower, and the fibre, presumably glassy, can be heat-treated to graphitize it; the properties of graphitized fibre are not mentioned. Kureda has now constructed a 120 ton plant (per annum output is presumably meant), and McLaren has listed several current applications including those depending on the low friction and wear associated with carbon fibre composites, and not requiring high stiffness. Some uses are distinctly unexpected: thus a large tonnage use is predicted for low-grade carbon fibre in carpets made from artificial fibres. Apparently litigation-conscious American carpet manufacturers are afraid of claims arising from deaths of patients with weak hearts who shuffle across highly insulating carpets, build up a charge and perish when they touch a door handle.

Another related development is solid glassy carbon, usually called vitreous carbon, which is made by pyrolysis of an unspecified polymer. The material was first studied in Japan, but large-scale development to the industrial stage has been undertaken by Plessey in Britain and it is now commercially available. Vitreous carbon has remarkable chemical stability, extremely low porosity, low friction, adequate electrical conductivity and high hardness, and this results in several novel applications, especially in the electronics and process industries and in spare part surgery. The properties of vitreous carbon have been surveyed in detail and compared with those of competing materials, and various uses have been described recently by F. C. Cowlard and L. E. Westmore (*Design Engineering*, 49, 52; March 1970).

PLANETARY SCIENCE

Smooth Surface of Mars

from a Correspondent

Lunar mascons and the surface properties of Mercury, Mars and Venus evoked lively discussions at the NATO Advanced Study Institute on the Moon and planets held at Newcastle upon Tyne from April 9–16 (early sessions were reported last week, *Nature*, **226**, 212; 1970). Dr W. L. S. Sjogren (Jet Propulsion Laboratory, California) dealt with the mascons, anomalous areas of locally higher gravitational field revealed by analysis of the orbits of Lunar Orbiter craft. He described the latest reduction based on a dynamic fit of all Lunar Orbiter data. Good correlations were found, with the prominent circular maria, Mare Orientale, shown to have a small positive anomaly surrounded by an annular negative anomaly.

Several interpretations were proposed for this. Dr M. J. O'Hara (University of Edinburgh) suggested that the mascons formed by the filling of depressions with the liquid phase of partially fused rocks. Because this phase concentrates the high density forming elements, subsequent solidification would produce the anomaly. The melt would rise to the surface if its density, while molten, were less than that of the surrounding rocks. Dr P. R. Bell (Oak Ridge National Laboratory) alternatively proposed that the surface of the Moon was originally molten due to an early "superluminous" phase of the Sun. Major impacts during the solidification of this layer produced pits into which the still molten fraction flowed, the fill and ejecta combining to form a positive gravity anomaly. Mare Orientale formed too late to have filled in this way. Professor H. C. Urey (University of California at San Diego) agreed that the surface must once have been molten, but pointed out that the presence of the mascons was difficult to reconcile with a molten or nearly molten interior. The interior must have a high degree of strength, for a subsidence of only one or two kilometres should eliminate the observed anomaly.

Dr G. H. Pettengill (Arecibo Observatory) reviewed recent radar studies of the terrestrial planets. Mercury seems closely to resemble the Moon in surface properties. Venus seems, on average, to be much smoother, though at least six anomalously rough areas have been mapped. Several small areas with heights of 1·5 to 2·0 km above the surroundings have been located, but there seems to be no large-scale relief. Mars seems to be even smoother than Venus, although there are large local variations in backscattering efficiency. There is a significant correlation between areas showing high echo specularity and those with low visual albedo, and there is considerable large-scale topographic variation in the northern tropics (the southern tropics have not yet been studied). Looking to the future, Pettengill predicted that, given adequate funds, it should be possible by 1974 to obtain radar echoes from the Galilean satellites of Jupiter, while by 1975 a 4 km radar resolution on the surface of Venus might be possible.

The description by Dr B. A. Smith (New Mexico State University Observatory) of the location of the satellite Phobos on several frames obtained from the Mariner 6 and 7 missions to Mars was a highlight of the proceedings. Phobos seems to be elongated (approximately 22·5 by 17·5 km) and to have a geometric albedo of about 0·065. If this figure is accurate, Smith pointed out, Phobos has the lowest albedo of any known object in the solar system, with the possible exception of some areas of the lunar surface.

When attention focused on the giant planets, the chief topic of interest was the magnetic field of Jupiter and the circulation of its atmosphere. Dr R. A. Stokes (University of Kentucky) warned that it is possible that a fluorescent or cyclic process could act in the atmosphere of Jupiter to produce infrared quanta with a non-equilibrium distribution. Some reported monochromatic temperatures may thus be significantly higher than the translational temperature of the source gas.

PROTEINS

Straightening out α-Keratin

from our Biomolecular Structure Correspondent

To be proposed independently by two Nobel laureates (and while both were in their model-building prime) might reasonably be taken to promise longevity for any structural hypothesis. Indeed, the coiled-coil structure for the α-fibrous proteins has been with us for more than seventeen years and the analysis on which it was based has influenced thinking on many topics in biomolecular structural analysis. Nevertheless, during the past few years there have been increasing signs of fundamental difficulties in the detailed fitting of the observed diffraction from the α-proteins with a coiled-coil model, and also indications that alternative models might account for the data reasonably well.

The most recent alternative to the coiled-coil model is due to Parry (*J. Theoret. Biol.*, **26**, 429; 1970) and brings together two proposals previously only considered separately. The first, due to Fraser and MacRae, suggests that each of the two or three α-helices which form the strands of the rope-like structure of α-proteins has a segmented structure. Each segment is a piece of undistorted α-helix typically ~20 Å long and the distortion resulting from folding of the strand is assumed to be confined to the few amino-acids in the region of the protein chain between segments. The second proposal derives from the work of Elliott and his co-workers, particularly the observation that in the paracrystalline phase of poly-γ-benzyl-L-glutamate in dimethylformamide the peptide backbone has the normal undistorted α-helical conformation, but the benzene rings at the ends of the side chains are arranged with quite different symmetry. The most striking feature in the diffraction from this structure is its similarity to that from α-proteins which have been identified as coiled-coils. This is particularly surprising because in the synthetic polypeptide structure the α-helical backbone in all the molecules is undistorted and all the molecules are parallel.

The segmented structure for the α-helical strands in the multi-stranded rope model for α-proteins allows each strand to assume a conformation reminiscent of a coiled-coil except that all the distortion from folding is confined to the part of the protein chain between segments. If the segment is not too long the favourable packing of side-chains in neighbouring α-helices, which provided the rationale for the original choice of the coiled-coil parameters, is largely retained in assemblies of segmented strands. The diffraction to be expected from such structures is in promising agreement with the observed "coiled-coil" data, and, possibly more important, that the intensity distributions in various regions of the diffraction pattern can be varied largely independently of each other. But this ability to adjust the calculated diffraction in such a regal way might be more a sign of the over-abundance of parameters in structures of this type than that the analyses are necessarily on a convergent track. Not only is the length of the segment largely undefined, so too is the number of strands in the multi-stranded rope and also their relative direction. Further allowance for the effect on the scattering of disordered material surrounding the molecules is fraught with difficulties although some progress is promised in this direction.

PROTEINS

To Catch an Enzyme

from our Molecular Biology Correspondent

AFFINITY methods of dipping into a mixture of components and extracting only the one with the right activity are, conceptually, at least as old as immunology, but their full power and versatility are only now being properly exploited. The principle is obvious enough: a ligand, for example, is immobilized and used to trap the protein for which it is specific, or vice versa. Affinity chromatography has, for example, been central to the work of Anfinsen and his colleagues on the chemical synthesis of staphylococcal nuclease. A terminal peptide fragment can be split from the chain, which will reassociate non-covalently with the residuum of the molecule to give a fully active enzyme. Chemical synthesis of the sequence corresponding to the terminal fragment gives an impure product because of side reactions, but the functional article is recovered from the mixture by passage through a column to which the residual protein is covalently attached.

The same principle has now been adapted to the capture of affinity-labelled fragments of the same enzyme. In affinity labelling of active sites, a bifunctional molecule is added to the protein, one end being a specific ligand (for example, inhibitor) that attaches at the active centre, the other a group of high chemical reactivity, which will react with any suitable side chain within reach. Digestion of the protein labelled in this manner then produces fragments, one of which contains the label and at least part of the active site or its environs. Givol *et al.* (*Biochem. Biophys. Res. Commun.*, **38**, 825; 1970) have hit on a way of isolating the fragment which circumvents the tedium of peptide separation procedures. The point is merely that the fragment still contains the specific ligand group, and may therefore be trapped on a column of the selfsame enzyme. As an example of this quite general strategy they give the following: pancreatic ribonuclease can be affinity-labelled with a derivative of uridine monophosphate with a phenyldiazonium-containing group substituted in the sugar. This reacts specifically with a tyrosine residue by the mononucleotide-binding site. The enzyme thus labelled is reduced, alkylated and digested with trypsin. The mixture of peptides is passed through a column of agarose beads to which ribonuclease is covalently bound through its amino groups by a now standard procedure that has been used to produce matrix-bound, insoluble enzymes. The mononucleotide end of the labelled peptide binds to the enzyme, all other peptides are washed through and the trapped ligand is flushed out with dilute alkali, pure and concentrated. A similar procedure has been used to prepare a peptide fragment from affinity-labelled anti-DNP antibody.

The application of the method to the study of active site peptides of staphylococcal nuclease is by Wilchek (*FEBS Letters*, **7**, 161; 1970). The specific inhibitor is deoxythymidine diphosphate, and the bifunctional version of this is substituted at the phosphate by a bromoacetyl-*p*-aminophenyl group. The bromoacetyl function is very reactive and attaches to lysines. After reaction and fragmentation by trypsin, the peptides are passed through a column of the nuclease bound to agarose, and only the labelled fragment sticks. A phenyldiazonium derivative has also been used in the same way, and reacts with a tyrosine residue. The peptide fragment was identified in terms of the position of its sequence in the chain, and it bears the label at Tyr-115, whereas the other reagent attaches to Lys-48 and Lys-49.

A more conventional application of affinity chromatography has been used by Gribnau *et al.* (*Biochem. Biophys. Res. Commun.*, **38**, 1064; 1970) for isolation of a protein inhibitor of ribonucleases. This comes from rat liver, and its partial purification by conventional methods has been a laborious matter. Pancreatic ribonuclease, anchored to a carboxymethylcellulose matrix (now a commercial product), was used to catch the specific inhibitor in the liver homogenate. After washing through the extraneous proteins, the enzyme–inhibitor complex was dissociated by high salt concentration. The product showed high activity, and was homogeneous by gel electrophoresis. Ribonucleases are the scourge of biochemists who operate in the general area of protein biosynthesis, and the rat liver inhibitor may prove to be of no small practical value.

POLYAMINES

Mysterious Functions

from a Correspondent

FORTY years after the election of S. O. Rosenheim to the Royal Society for discovering spermidine, and clarifying both its structure and that of spermine, the functions of these compounds and of their precursor, putrescine, are beginning to be understood. Recent advances in their physiology and biochemistry were discussed at a conference held on April 9 and 10 by the New York Academy of Sciences.

Drs A. B. Pardee and M. Inouye (Princeton University) described how putrescine and spermidine act in the synchronization of the division of amino-acid-deficient bacterial mutants. Dr W. K. Maas (New York University) described how division of putrescine-requiring strains of *E. coli* was selectively arrested in the absence of exogenous putrescine and spermidine. The genes for several steps in putrescine biosynthesis are clustered, suggesting that there is an operon for this function. Dr D. R. Morris (University of Washington) described the network of constitutive and inducible enzymes of putrescine biosynthesis in *E. coli*. The production of putrescine is subject to coarse controls of repression and derepression, and to fine controls of feedback inhibition and substrate availability. Dr S. S. Cohen (University of Pennsylvania) described the production of abnormally large amounts of spermidine in relaxed bacteria and of putrescine in organisms deficient in potassium ions. The latter phenomenon, which also occurs in plants, although involving different mechanisms of enzyme imbalance, was suggested by Dr T. A. Smith (Long Ashton Research Station, Bristol) to indicate the existence of a homoeostatic mechanism operative in acidic soils or those deficient in potassium ions.

Dr L. Stevens (King's College, London) showed spermidine and spermine to be bound to cellular ribosomes. These polyamines were found by Cohen in tRNA isolated at low ionic strength. Detection in tRNA involved a new sensitive fluorometric method described initially by Dr A. S. Dion and Dr G. J. Herbst (University of New Hampshire) in their work on the development of *Drosophila*. Dr J. R. Fresco (Princeton University) described the conversion of an inactive form of tRNA to an active configuration by three molecules of spermidine per molecule of the nucleic acid. Dr P. S. Leboy (University of Pennsylvania) indicated that polyamines could activate a tRNA methylase.

A developmental event involving an increase in RNA always parallels synthesis of spermidine. Dr A. Raina (University of Helsinki) and Dr S. H. Snyder (Johns Hopkins University) have shown that the early synthesis of spermidine and RNA in regenerating rat liver is anticipated by the specific stimulated synthesis of ornithine decarboxylase and of its product, putrescine. Dr A. E. Pegg (Courtauld Institute, London) and Dr H. G. Williams-Ashman (University of Chicago) have solved the problem of spermine synthesis by demonstrating a double-headed enzyme which decarboxylates S-adenosyl methionine in the presence of putrescine, and then transfers an aminopropyl moiety to putrescine and spermidine to form spermidine and spermine, respectively.

Dr C. M. Caldarera (University of Bologna) demonstrated similar correlations in embryonic systems. These enzymes are reported to have the shortest half lives (less than thirty minutes) of any described in mammalian tissues; this seems to relate to their rapid disappearance when rapid growth ceases. The finding that neither enzyme is produced in anucleolate *Xenopus laevis* suggests a possible integration of nucleolar function in the elaboration of both the ribosomal RNA necessary for growth and the polyamines tied to that RNA.

PROTEINS

Links in the Tissue

from a Correspondent

MEMBERS of the Collagen Club met on April 3 at the Meat Research Institute, Langford, to discuss the chemistry of the cross link in connective tissue proteins. In collagen and elastin intermolecular cross links in particular confer valuable physical properties. Dr S. M. Partridge (MRI) spoke about cross linkages in elastin. It was Partridge and his colleagues, of course, who in 1963 isolated the amino-acids desmosine and isodesmosine from hydrolysates of bovine ligamentum nuchae and, later, of aorta. On the basis of analytical data and electron microscopy, Partridge suggested that a suitable model for elastin was an arrangement of hydrophobic globular "pro-elastin" particles of mass 67,000, connected to each other by desmosine molecules. Such a model would account for results obtained by Dr D. P. Mukherjee (Massachusetts Institute of Technology), who has examined the visco-elastic properties of elastin. Dr S. O. Anderson (University of Cambridge) had studied heat changes of elastin on stretching and his findings could also be explained by Partridge's proposed particulate structure.

In spite of considerable differences in chemical composition and properties between elastin and collagen, Dr R. A. Grant (ARC Institute of Animal Physiology, Babraham) claimed evidence for similarities in the primary structure of these two proteins. When heat-shrunk collagen was treated with elastase and the released neutral peptides were subjected to fingerprinting, the pattern obtained resembled that of elastase-treated elastin, but was different from that of elastase-treated ovalbumin and of bovine serum albumin. Elastase-treated collagen α_1 and α_2 chains also gave similar patterns. Grant suggested that elastin and collagen contained certain common amino-acid sequences.

Although various interchain cross links have been postulated for collagen, those derived from lysine aldehyde are well established. Dr A. J. Bailey (MRI) provided evidence that this type of linking occurred intermolecularly. As in elastin, a lysine residue was first converted by amine oxidase to aldehyde. The aldehyde could react with a group of another residue suitably positioned to form an interchain cross link. Various cross links could therefore be produced, some of which were labile and would not survive hydrolysis conditions. If, however, they were reduced with borohydride the cross links were converted to stable derivatives. Hydrolysis followed by amino-acid analysis would reveal the presence of a new amino-acid derived from the reduced cross link, which if identified would provide evidence for the nature of the original cross link. If tritiated borohydride was used for reduction, measurement of radioactivity in the amino-acid chromatogram peaks would provide a measure of the number of residues involved in cross linking. As an example, the production of hydroxylysinonorleucine after reduction indicated that the native cross link was formed by a hydroxylysine-derived aldehyde condensing with an amino end group of a lysine side chain to form a Schiff base.

Dr L. J. Fowler (MRI) described experiments which could provide important information about the biosynthesis of lysine-derived cross links. In lathyritic animals, collagen has a deficiency of cross links when analysed by the procedure described by Bailey. However, when bone collagen from lathyritic chickens was incubated with extracts of healthy chicken tibia and femur, a large increase of lysine aldehyde-derived constituent was observed, presumably due to amine oxidase extracted from the normal tissues. Similar results were obtained when lathyritic pig collagen was incubated with extracts of normal pig tissue. This type of experiment could be used for examining inhibition or enhancement of amine oxidase activity.

When collagen is irradiated with ultraviolet light it fluoresces bluish white, and some think that this is due to cross links derived from tyrosine residues. Mr C. McDevitt spoke about his work with Dr W. G. Armstrong at the Royal Dental Hospital, directed at isolating and examining this fluorescent material. Starting from ox femur collagen, they fractionated the fluorescent material in alkaline hydrolysates, but it was not dityrosine (which is fluorescent and present in resilin) because it differed in spectral characteristics and chromatographic properties. Tyrosine was probably involved, however, because when rats were fed on radioactive tyrosine, the fluorescent fraction from their collagen was radioactive and resembled that from the ox femur. The latter seemed to contain acidic groups and its chemical properties and mass spectra suggested that it was a derivative (probably pyrophosphate) of thiamine. It was not possible at this stage to say whether the thiamine was covalently linked with the collagen, but it seemed to be fairly firmly bound.

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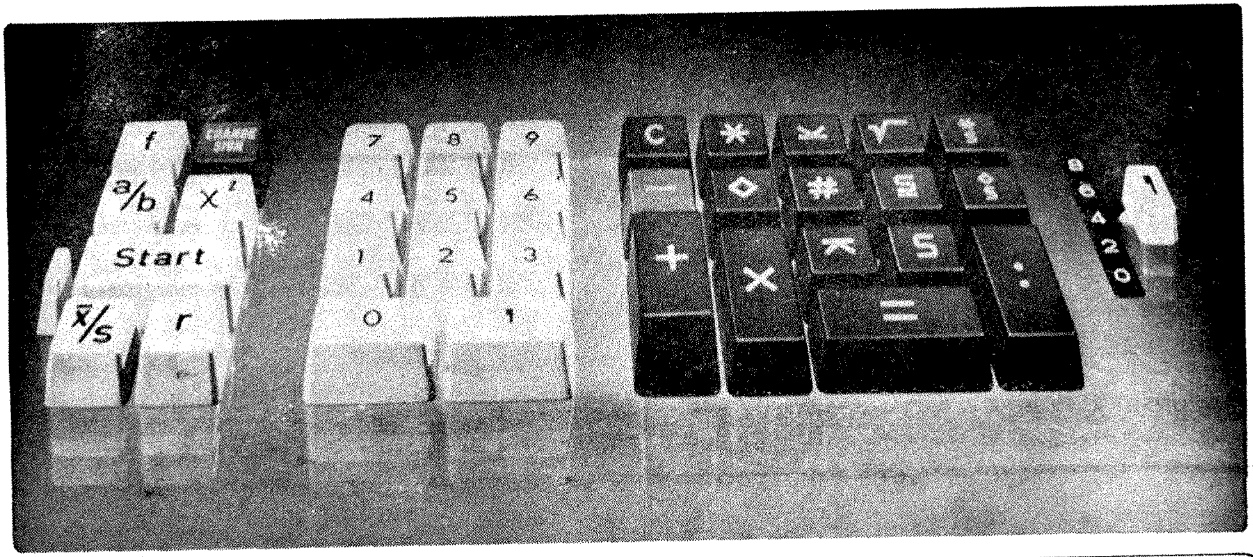

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Multiple-Beam Interference Microscopy of Metals

S. Tolansky *Royal Holloway College, University of London, Egham, Surrey, England*

March 1970, x + 148 pp., 45s. (£2.25) SBN 12.692650.6

This monograph is devoted to the use of multiple-beam interferometry for the study of metals, especially those used in engineering technologies. It gives the "know-how" of many years' study and use of the technique which will enable metals scientists and engineers to exploit the technique to advantage.

Contents Principles of multiple-beam interference. Multiple-beam localized fringes. Preparation of reflecting films. Microscope arrangements. The matching flats and fringe definition. Evaluation of topographic features. Efficiency of silver contour. Surface roughness: interference contrast. Hardness measurement. Thickness of thin films. Some applications. Historical object—Barton's button. Some studies on silicon carbide crystals. References. Appendix: fringes of equal chromatic order. Index.

Biochemical Society Symposium Number 29

Natural Substances Formed Biologically from Mevalonic Acid

Edited by T. W. Goodwin *Department of Biochemistry, University of Liverpool, England*

February 1970, x + 186 pp., 55s. (£2.75) SBN 12.289865.6

The discovery of mevalonic acid as a specific precursor of terpenoids revolutionized this field of biochemistry. This book represents a stocktaking, by world-famous authorities, of recent developments. It is essential reading for all research workers in the field and for senior undergraduate biochemistry students.

Contents Chairman's opening remarks. Chemistry of mevalonic acid. Conversion of mevalonic acid into prenyl hydrocarbons as exemplified by the synthesis of squalene. Enzymatic studies on the oxidative cyclizations of squalene. Sterol biosynthesis. Nature and distribution of terpene quinones. The biosynthesis of terpenoid quinones. Polyprenols. Biological function of terpenoid quinones. Terpenoids in insects. Biosynthesis of terpenoid alkaloids. Chairman's concluding remarks. Author index. Subject index.

Society for Applied Bacteriology Technical Series No. 4

Automation, Mechanization and Data Handling in Microbiology

Edited by Ann Baillie *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, England*

and R. J. Gilbert *Food Hygiene Laboratory, Central Public Health Laboratory, London, England*

March 1970, xvi + 234 pp., 65s. (£3.25) SBN 12.073650.0

This volume brings together a number of time- and labour-saving techniques from different disciplines and indicates their potential usefulness, describing possible applications of automation, mechanization and data handling techniques to microbiology. This book will be of interest to microbiologists, clinical pathologists and students of microbiology and biochemistry.

Contents Computer identification of bacteria. A computer-linked data processing system for routine hospital bacteriology. Computer use in information and data handling. The application of some automation and data handling techniques to the evaluation of antimicrobial agents. Automated reading of large assay plates. Identification of bacteria with the aid of an improved information sorter. Applications of the Coulter Counter in microbiology. Fully automated high speed amino acid analysis. An automated technique for the determination of ammonia produced by bacteria. The POMEC—an apparatus for growing dense cultures of pathogenic micro-organisms. An automatic growth recorder for microbial cultures. Control of oxygen tension using the Mackreth electrode. Multiple inoculation technique for rapid identification of bacteria. A multiple dropper for micro-titration apparatus. Examples of mechanization in microbiology media service sections. Automatic methods in diagnostic bacteriology. Author index. Subject index.

The Yeasts, Volume 3: Yeast Technology

Edited by A. H. Rose *School of Biological Sciences, Bath University, Bath, England*

and J. S. Harrison *Research Department, The Distiller's Co. (Yeast), Ltd., Epsom, Surrey, England*

April 1970, xiv + 590 pp., 150s. (£7.50) SBN 12.596403.X

Yeasts have been associated more intimately than any other group of micro-organisms with the progress and well-being of mankind. In this volume specialists from universities, research and industry deal with yeast technology, each giving a detailed and balanced view of his or her own field of interest.

Contents Introduction. Yeasts in wine-making. The role of yeasts in cider-making. Brewer's yeasts. Saké yeasts. Yeasts in distillery practice. Baker's yeasts. Food yeasts. Yeasts as spoilage organisms. Miscellaneous products from yeasts. Author index. Subject index.

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Control and Use of Scientific Information

by
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Eltham,
London SE9

Since 1966 Unesco and the International Council of Scientific Unions have been looking into the feasibility of a world science information system. Dr Coblans, a member of the committee concerned, gives a progress report on the project at a crucial stage in its history.

THE concept of a world centre conserving all documents and serving everyone has long been a recurrent theme. Even though there have been brave attempts at its realization, it was clearly Utopian. A typical attempt was the International Catalogue of Scientific Literature for the twentieth century, sponsored by the Royal Society and abandoned in 1922 after a subject catalogue for the literature of the first fourteen years of the century had appeared. The attempt to establish a world bibliographical centre for "education, science and culture" by Unesco in Paris in the late nineteen-forties did not survive the first stages of planning and implementation.

Nonetheless, Unesco by its constitution and purpose has a basic responsibility in this field, and it is therefore right and proper that, jointly with the International Council of Scientific Unions (ICSU), it should be investigating the feasibility of UNISIST, a phonetic invention to symbolize the part which the United Nations (UN . . .) should take in promoting a world-wide system (. . . SIST) of information, both scientific and technical (. . . IST). It should be stressed that this time there is no suggestion of a vast central bureau, but rather the coordination and interconnexion of existing networks, chiefly national, based on voluntary cooperation and the standardization essential for the exchange of documentation and information.

At the General Conference of Unesco held in 1966, a common programme was approved under the title "Joint ICSU–Unesco project in the communication of scientific information and on the feasibility of a world science information system". A working party met shortly afterwards to define the broad outlines and the implementation of the proposed study. As a result, a central committee* was created and this has met four times since December 1967. The project has now reached a crucial point, for the draft report, prepared by Dr J.-C. Gardin, was considered and accepted at the fourth session which was held in December 1969.

Working Groups

The central committee has worked principally through six working groups to which specific tasks have been delegated: evaluation, compression and organization of scientific information; indexing and classification; bibliographic descriptions; language problems; research needs in documentation; scientific information in developing countries. In addition, the committee has had the benefit of the comments and criticisms of an advisory panel of nine representatives (present as observers) of large working services such as MEDLARS, Euratom, the American Chemical Society and the Information Service in Physics, Electrotechnology, Computers and Control (INSPEC).

Of all these working groups, that concerned with bibliographic descriptions is the most important at present and has been the most productive during the two years of its operation. The reason is simple—there can be no real world transfer of scientific information to the general benefit of all countries, whether industrially advanced or developing, unless there is a minimum of standardization in the "language" of communication. In certain topics where a meta-language of symbols exists (for example, in mathematics, organic chemistry, classical taxonomy) the literature of science is truly international, but even here the problems of machine storage and print-out remain to be solved economically, although prospects are very encouraging. But, even if the content of a scientific paper is thus understood by fellow scientists the world over, its identification and description as a bibliographical unit are at present based on a somewhat chaotic set of conventions arising from national and language practices that are chiefly historical in origin. Whatever else the use of computer methods in documentation has achieved, it has made it abundantly clear that there can be no real economies and progress unless magnetic tape stores and the related computer-aided printing are reasonably compatible and interchangeable and therefore exchangeable.

This essential standardization is the responsibility of the International Organization for Standardization (ISO), a federation of national standardizing bodies which makes international recommendations which are subsequently translated into national standards. Inevitably ISO can only work slowly because its recommendations must be circulated to all member states for comment. What emerges is all too often a compromise, theoretically defensible, but not used much by the major services in abstracting and indexing or even less by the scientific press.

The working group on bibliographic descriptions started from the other end, and has looked at the daily practice of the large existing services, particularly those within the ambit of the ICSU Abstracting Board, and even more significantly those that are being computerized. By bringing these together and negotiating in areas where agreement on small changes is not difficult to achieve, UNISIST has begun to have some success. Once these standards have been accepted by the large services, chiefly international in scope, there is every chance that ISO will manage to promulgate them as recommended international standards.

At the meeting of UNISIST last December, this work was reported to be fairly well advanced in three areas. First, there is the question of essential information for the control of periodical literature (some fifteen elements). In each case this requires identification of the periodical, the specific issue, the paper within the issue, the "author" responsible for the publication and the language. This involves the accepted abbreviations of words in periodical titles in all languages for each periodical title. Thus a UNISIST list of abbreviations will have to be kept up to date. Similarly a world register for scientific and technical periodicals, a very large undertaking, is proposed. It would provide a machine file of periodical titles and standard serial numbers (SSN) on the analogy of the widely accepted standard book numbers (SBN). This would make possible a unique identification code for all documents (papers, reports and so on) with information value. The practices of citing the names and affiliations of authors, for most of the countries publishing literature, have been analysed and a set of recommendations will be tested for suitability.

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* Chairman: Dr Harrison Brown (foreign secretary, US National Academy of Sciences, Washington, representing ICSU). Members: Dr B. W. Adkinson (USA), Eng. N. B. Arutyunov (USSR), Professor G. A. Boutry (France), Dr H. Coblans (UK), Dr H. T. Hookway (UK), Professor H. Kaiser (Federal Republic of Germany), Professor M. Kotani (Japan), Professor A. I. Mikhailov (USSR), M. P. Piganiol (France), Dr F. A. Stafleu (Netherlands). Unesco representative: Dr A. Pérez Vitoria, later replaced by Dr A. Wysocki. The original membership also included Dr P. Lazar (Hungary) and Dr C. Sherwin (USA), who resigned for various reasons and were replaced by Professor A. Avramescu (Rumania) and Dr Milton Harris (USA). Observers: the sessions have been attended by representatives from more than twenty international organizations, all directly involved in documentation and information transfer.

Second, on the question of transliteration, the ISO standard for Cyrillic alphabets is internationally accepted for scholarly use, but the diacritical marks are not handled easily on computers. By agreement with the All-Union Institute of Scientific and Technical Information (VINITI) in Moscow, an international standard avoiding the use of diacriticals has been proposed. Similarly, conventions have been worked out for the special letters used in Germanic and other languages.

The third line of advance concerns character sets and encoding systems. The importance of compatibility for merging duplicate tapes in information retrieval systems and the computer-aided printing of lists is widely understood, but remains a serious obstacle. It was agreed to start from a basic set of forty-five characters (Roman capitals, ten digits and nine additional characters). An inventory of additional characters used by most large services is being drawn up. Each character not in the basic set will be encoded by the use of two or more of the basic characters. Another important, but somewhat intractable, standard remains to be studied—the sorting order of the character set. The recommendations and standards I have described have received agreement from all the member services of the ICSU Abstracting Board, as well as several international organizations, and they propose to implement them progressively. This would have seemed Utopian even a few years ago.

Voluntary Cooperation

The other working groups by their nature were not able to act so effectively; their function has been to analyse, to define the problems and to sound out informed opinion at all levels on as international a scale as possible. In a sense the draft report prepared by Gardin has provided a statement of the present position and a synthesis of what has emerged from the activities of the working groups and the central committee. The assumptions of such a world science information system are summarized as follows in the report.

(*a*) Scientific and technical information, as embodied mainly in the more formal printed sources, is the property of all nations; (*b*) this printed literature, published or unpublished, can be considered for this reason as a collective store, from which information is to be extracted, organized, processed, evaluated, re-packaged, etc., in a variety of ways, by the concerted effort of all countries; (*c*) these operations in the "transfer chain" are best performed through a worldwide division of labour between all parties, with due consideration but no necessary subjection to existing geopolitical divisions; (*d*) the output from each party, at any stage of transfer, can then be made available as input to other parties, subject to the acceptance of common standards; (*e*) such standards are well within our reach, provided proper mechanisms be set up to hasten their definition, ratification and implementation by all parties.

The central committee has tried to harmonize the varying needs and assign priorities for such a decentralized system of voluntary cooperation among existing and future services in various countries and regions, languages and disciplines. Such an inventory would include, first, bibliographic and machine standards. These would be internationally accepted and used, with a central coordinating office to maintain them and develop new ones throughout the UNISIST community and speed up formal ratification through ISO. Second, there would be specification of subject content and information retrieval. This is the most complex problem of documentation because it demands much more research into the use of general classifications, the evaluation of indexing languages, the construction of compatible thesauri and standard lists of subject headings. Third, the inventory would include linguistic and semantic problems. These arise in the machine processing of scientific texts especially for automatic indexing; in on-line "dialogue" between user and computer; for indexing by free text searching for words; and, most difficult of all, for machine translation. And, fourth, would be the needs arising from the fact that if developing countries are to benefit from what UNISIST can potentially offer, they must be able to reach a basic level of requirements. This would include at least two types of resources, books, periodicals and so on, and reference tools for their utilization located in professionally staffed libraries, and a nucleus of technical manpower at a level appropriate to the size and development objectives of the country. To help in providing these needs, and above all in the training of the necessary staff, the United Nations Development Programme will be asked to make funds available for the creation, under international control, of several regional centres as pilot projects in carefully selected areas. These would act as clearing houses for local and external documents; as centres for the education and training of information personnel and also the users of information; as the focal points for the international exchange of information and the exploitation of mechanized stores in the form of duplicate magnetic tapes.

While there was general agreement on the basic principles of the draft report, there remain several partly resolved questions, which are inherently difficult. The principal difficulty concerns national languages, the subject scope of UNISIST and the form of its organizational structure when it comes into being.

Language Problems

Governmental international organizations tend to work in the four or five languages which dominate international communication and discourse. This is even more marked in some of the natural sciences; for example, in physics and chemistry, comprehensive abstracting services are more and more appearing only in English, Russian and French. Here the hard facts of economic life are determinant. On the other hand, several national representatives feel strongly that language should not be used as a sort of "colonial" weapon. They argue that, because language is a fundamental component of each national culture, Unesco should promote the development of national languages, particularly in African and Asian countries. This clearly complicates the problems of UNISIST, which is already faced with the negative effects of language barriers on the flow of information.

An attractive proposal has been that each developing country, while fostering its own local language, should choose a major language as its "language of transfer".

UNISIST has evolved from a joint initiative of Unesco and ICSU and as such has stressed the natural sciences as the starting point, hoping to move progressively into the technological fields. Strong voices are insisting that from the beginning UNISIST should cover not only the pure but also the applied sciences, for from the point of view of information they cannot really be disentangled. Science and technology are being more and more affected by social and economic considerations as science policy and management become accepted as national imperatives. Therefore the separation of pure, applied and social sciences, especially from the point of view of information, is no longer realistic and efficient.

The third question is clearly controversial: what kind of organization can handle—coordinate and lead—so varied a conglomeration as governments, non-government organizations, learned societies, universities, industrial units? The draft report convincingly shows that UNISIST is feasible and necessary. Therefore the next steps must be to find the financial resources and create the administrative machinery for implementing and maintaining such a world-wide system of cooperation. To do this, Unesco, jointly with ICSU, is planning an international conference to be held probably in the last quarter of 1971 to bring together about 300 scientists, information specialists and government officials responsible for research and information policy. For this conference the final report on feasibility will be the working paper.

Administrative Structure

One suggested administrative structure, on the model of the very effective Intergovernmental Oceanographic Commission, is an International Commission for Scientific Information. Such a commission combining government, professional and user interests would indeed be a breakthrough. For the first time there would be a central steering body capable of evaluating, interpreting and then presenting to governments and others what can and must be done now, and furthermore with the executive strength to act. So far we have had no such overall and accepted body with the status and the power to do this. There are numerous international organizations each active in its own way: Unesco organizing documentation centres for developing countries, ISO promoting standards for documentation, WHO and FAO taking some responsibility for documentation in certain subject fields, OECD stimulating science information policy at the national level, ICSU coordinating abstracting services, FID furthering documentation at the professional level and so on. No organization has had the mandate and the resources to steer these multifarious activities and act on limited agreement. Nowhere are these problems seen and acted upon as a whole in all their intricate inter-relationships. By and large, the result has been an ineffectual Babel and therein lies our information crisis!

Characterization of Ovine Hypothalamic Hypophysiotropic TSH-releasing Factor

by
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Evidence is presented for the homogeneity of a preparation of hypothalamic TRF of ovine origin and its molecular structure has been established as 2-pyrrolidone-5-carboxylyl-histidyl-proline amide. Synthetic L-2-pyrrolidone-5-carboxylyl-L-histidyl-L-proline amide has physicochemical and biological characteristics which are quantitatively and qualitatively indistinguishable from those of the natural substance isolated in this laboratory.

THE existence of hypothalamic thyrotropin (TSH) releasing factor was demonstrated in 1962 (ref. 1). Recently[2] we obtained a highly purified preparation of hypothalamic TRF (acetate) of ovine origin according to the purification sequence summarized in Table 1 and demonstrated by analysis of a 6 M HCl hydrolysate that it contained as much as 81 per cent of its molecular weight in terms of the three amino-acids, glutamic acid, histidine and proline in equimolar ratios (theoretical contribution of the three amino-acids for a tripeptide acetate = 86 per cent). No free N-terminus could be demonstrated in this preparation of ovine TRF by dansylation. We subsequently reported that fully synthetic tripeptides of the generic structure PCA-His-Pro-R (PCA = 2-pyrrolidone-5-carboxyl) but not Glu-His-Pro-R, had TRF activity qualitatively indistinguishable from that of natural TRF. The specific activity of the tripeptides increases in the order $R = OH < OCH_3 < NH_2$, PCA-His-Pro-NH$_2$ having a biological activity not statistically different from that of natural TRF obtained as above[3-5]. Furthermore, the physicochemical characteristics of the various synthetic tripeptides, and particularly of PCA-His-Pro-NH$_2$, were closely related to those of natural ovine TRF. On the basis of low and high resolution mass spectrometric studies of highly purified ovine TRF and of a pure preparation of synthetic PCA-His-Pro-NH$_2$ (ref. 6), we have proposed that the molecular structure of hypothalamic ovine TRF is PCA-His-Pro-NH$_2$ (ref. 7).

We report here the detailed evidence on which this statement was based as well as information on (1) the nuclear magnetic resonance (NMR) spectra of pure ovine TRF and the synthetic tripeptide-amide; (2) quantitative amino-acid and ammonia analyses of natural ovine TRF and the two synthetic tripeptides PCA-His-Pro-OH and PCA-His-Pro-NH$_2$; (3) the biological activity of pure

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† Department of Biochemistry, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

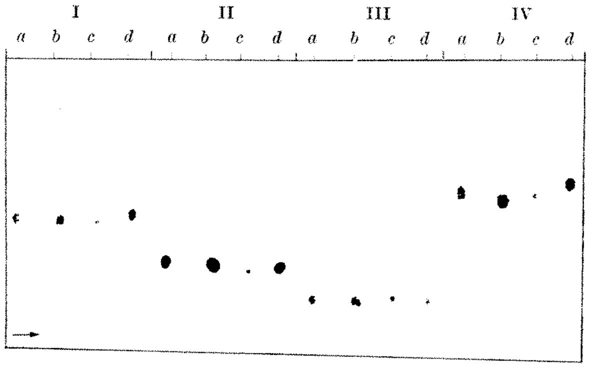


Fig. 1. Thin-layer chromatography of (*a*) 5 μg PCA-His-Pro-NH_2 prepared by total synthesis; (*b*) a mixture of 5 μg PCA-His-Pro-NH_2 prepared by total synthesis and 2·5 μg natural ovine TRF; (*c*) 2·5 μg natural ovine TRF; and (*d*) a mixture of 2·5 μg PCA-His-Pro-NH_2 prepared by total synthesis and 2·5 μg PCA-His-Pro-NH_2 prepared by ammonolysis of the methyl ester of PCA-Glu-His-Pro-OH. Thin-layer chromatography was performed on silica gel G (Eastman 'K 301 R2') in the systems: I, *n*-butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1); II, *n*-butanol–acetic acid–water (4 : 1 : 5); III, *n*-butanol–ethyl acetate–0·2 M aqueous NH_4OH (1 : 1 : 2); IV, collidine–ethanol–diethylamine–water (220 : 100 : 1 : 80). The upper phase was used in the case of two phase systems.

ovine TRF compared with synthetic PCA-His-Pro-NH_2. The new data support our earlier conclusions and prove that the tripeptide derivative PCA-His-Pro-NH_2 prepared by ammonolysis of the methyl-ester of PCA-His-Pro-OH[4,5] or prepared by total synthesis[6] has characteristics practically identical to those of natural ovine TRF and a biological activity which is not statistically different.

Physicochemical Characterization

Mixtures of 2·5–5·0 μg aliquots of natural ovine TRF and synthetic PCA-His-Pro-NH_2 move as a single spot as stained by the Pauly reagent in several chromatographic systems (Fig. 1).

For the examination of infrared spectra, 50 μg of natural ovine TRF (acetate) and of synthetic PCA-His-Pro-NH_2 (acetate) was deposited as thin films on the window of an 'Irtran' cell; spectra were observed in a Beckman 'IR-9' spectrometer. The spectra of the two materials (Fig. 2) are identical at all wavelengths with the exception of a peak at 1,110 ± 9 cm^{-1} (mean of six independent experiments) in the spectrum of synthetic PCA-His-Pro-NH_2 which is displaced to 1,121 cm^{-1} (mean of three independent experiments) in the spectrum of natural ovine TRF. This minor discrepancy, although observed with two different batches of TRF, is not statistically significant. Studies with a series of synthetic analogues of the generic structure PCA-His-Pro-R have indicated the possibility of producing such minor shifts by changing the *p*H of the solution(s) deposited on the 'Irtran' cell; the presence of traces of metal(s) chelated by either peptide may also be a possible explanation. We consider the similarities of the two spectra to be sufficient to indicate identity.

Table 1. SEQUENCE OF PURIFICATION FOR ISOLATION OF OVINE TRF

Stage	No. of fragments × 1,000*	Weight†	TRF units/mg‡
1. Lyophilized sheep hypothalamus	294	25 kg	
2. Alcohol–chloroform extract	294	294 g	1
3. Ultrafiltration 'UM-3'	294	71 g	3
4–5. Gel filtration, 'Sephadex *G*-25', 0·5 M acetic acid, 2X	286	16 g	16
6–7. Partition chromatography, 0·01 per cent acetic acid–*n*-butanol–pyridine (11 : 5 : 3), 2X	280	246 mg	800
8–9. Adsorption chromatography, Norit/H_2O–ethanol–phenol, 2X	275	4·2 mg	30,500 (1,100–200,000)
10. Partition chromatography, *n*-butanol–acetic acid–H_2O (4 : 1 : 5, upper phase)	273	2·0 mg	58,500 (12,500–150,000)
11. Repeat 10	270	1·0 mg	57,000 (12,000–124,000)

* Number of hypothalamic fragments available at each step in the purification sequence; the continuous decrease in number from step 4 to 11 results from the removal of aliquots for pilot studies, repeated bioassays, and so on.

† From step 3 to 11, the weights correspond to those of the TRF-active fractions at each step.

‡ Specific activity (TRF U/mg) with 95 per cent confidence limits in multiple four-point assays.

Low resolution mass spectrometry was performed on 20–40 μg samples of ovine TRF from stage 11 (Table 1) and of synthetic PCA-His-Pro-NH_2 which were methylated by treatment with an excess (50 μl.) of a solution of diazomethane in diethyl ether (3 × 5 min). Similar aliquots were also derivatized by treatment with 50 μl. of a 1 : 1 mixture of trifluoroacetic anhydride and trifluoroacetic acid (1 h, room temperature). Figs. 3 and 4 illustrate the low resolution spectra of the products. All samples were volatile in low resolution mass spectrometry (LKB 9000, direct probe, $\leq 10^{-6}$ torr) at probe temperatures ranging from 150°–200° C. Several mass spectra for each sample (seven in the case of TRF derivatives), recorded at various temperatures along the programmed thermal gradient, yielded identical fragmentation patterns corresponding to a single chemical entity. Combined with other evidence (quantitative amino-acid content[2], nuclear magnetic resonance spectra, thin-layer chromatographic data) these results allow us to claim the preparation, for the first time, of a hypothalamic hypophysiotropic releasing factor.

The mass spectra of the same derivatives (trifluoroacetylated or methylated) of natural ovine TRF and synthetic PCA-His-Pro-NH_2 are essentially identical (Figs. 3 and 4). No molecular ion was observed in any of these spectra; similar fragments were observed in the low resolution spectra of each derivative of natural TRF and synthetic PCA-His-Pro-NH_2. Ions were observed corresponding to the following structures: PCA, *m/e* 84; PCA + CH_2, *m/e* 98; His, *m/e* 94; His + CH_2 *m/e* 108; Pro minus 3H, *m/e* 93; Pro-NH_2 minus 3H, *m/e* 110 (unsaturated proline fragments such as pyrrole-2-carboxamide possibly produced by pyrolysis); $CONH_2$, *m/e* 44; PCA-His, *m/e* 221; His-Pro-NH_2 minus 2H, *m/e* 248. Other fragments, *m/e* 234, 235 and 249, could arise from PCA-His, His-Pro-NH_2 or also, as Schally *et al.* mentioned in the case of a preparation of porcine TRF[8], from the diketopiperazines of histidine and proline.

The elemental composition of all these fragments, except ion *m/e* 221 which was too weak to give a readable trace on the photoplate (Ilford 'Q2'), was confirmed on the methylated derivatives of TRF and synthetic PCA-His-Pro-NH_2, using high resolution mass spectroscopy ('CEC 21-110') (Table 2).

For nuclear magnetic resonance (NMR) spectrometry,

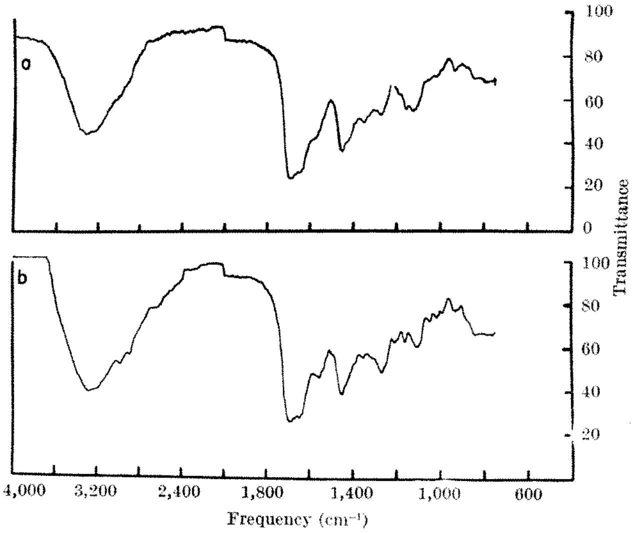


Fig. 2. Infrared spectra of (*a*) isolated ovine TRF and (*b*) synthetic PCA-His-Pro-NH_2.

0·8 mg of ovine TRF acetate (stage 11, Table 1) was dried in a 5 mm NMR tube and redissolved in 200–300 µl. ^2H_2O. The NMR spectrum of this preparation was obtained using a 100 MHz spectrometer (Varian 'HA-100') with time averaging by coupling to a computer of average transients ('C-1024'). Fig. 5*a* shows the spectrum, in comparison with a single sweep spectrum (Fig. 5*b*) of 25 mg synthetic PCA-His-Pro-NH_2 (acetate) as a solution in a similar volume of ^2H_2O using the same spectrometer. In the regions that can be compared, the spectra of the two compounds are practically identical. The singlet at 3·5 p.p.m. present in the TRF spectrum but absent in that of the synthetic compound may represent a minor impurity, but could also arise from a spinning side-band of the peak for water. The slight differences in the location of the maxima in the 7–9 p.p.m. region, presumably from the histidine ring protons, can be explained by the *p*H sensitivity of these bands in histidine NMR spectra[9].

Histidine and ammonia contents were determined in TRF and synthetic analogues, and the results are given in Table 3. Quantitative analyses of 6 M HCl hydrolysates of ovine TRF, synthetic PCA-His-Pro-OH, synthetic PCA-His-Pro-HN_2 and unhydrolysed TRF were obtained using a Beckman-Spinco modified model '120C'. The figures obtained for ovine TRF correspond to the presence of an amide group on the C-terminal proline.

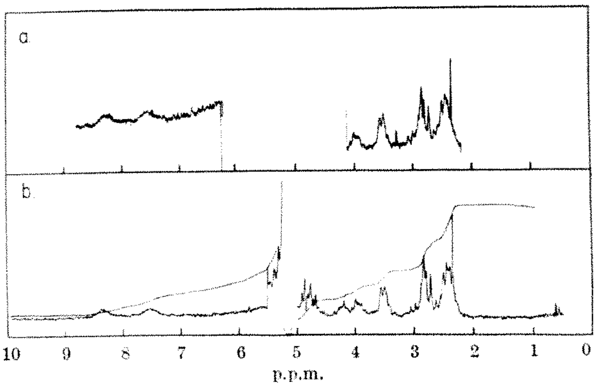


Fig. 5. Proton magnetic resonance spectra (100 MHz) of (*a*) ovine TRF acetate in ^2H_2O, time-averaged for 276 scans, 500 s/scan, 500 Hz sweep width in the regions shown, lock signal internal H^2HO (5·14 p.p.m. referenced to external capillary of tetramethylsilane in a separate experiment); (*b*) synthetic PCA-His-Pro-NH_2 (acetate) in ^2H_2O, single scan 1,000 s, sweep widths 1,000 Hz; referenced to external capillary of tetramethylsilane. Both spectra were obtained at 31° C.

Biological Activities

Using an *in vivo* mouse assay[5], six independent four-point assays of homogeneous natural ovine TRF gave a specific activity of 55,000 ± 9,900 U/mg (see ref. 10 for the definition of the TRF unit); five independent four-point assays of synthetic PCA-His-Pro-NH_2 gave a specific

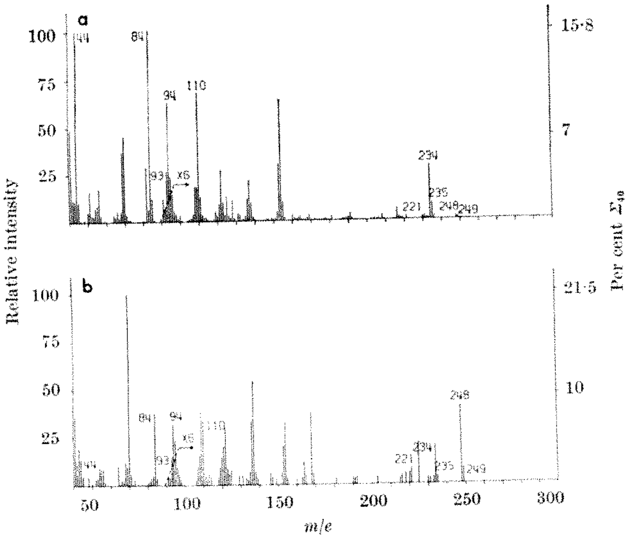


Fig. 3. Low resolution mass spectra of trifluoroacetylated ovine TRF (*a*) and synthetic PCA-His-Pro-NH_2 (*b*).

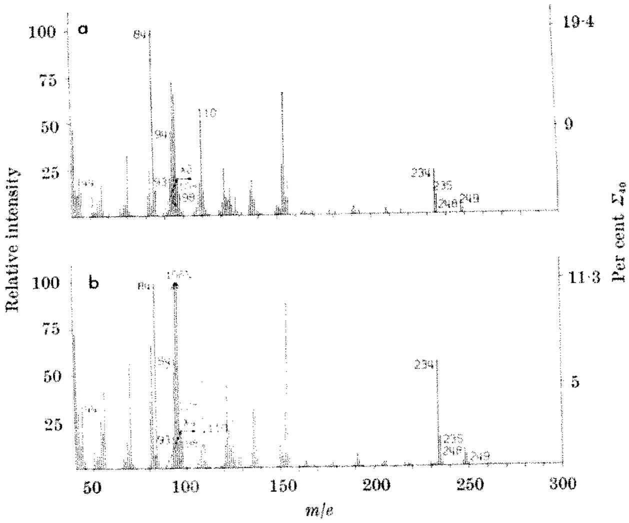
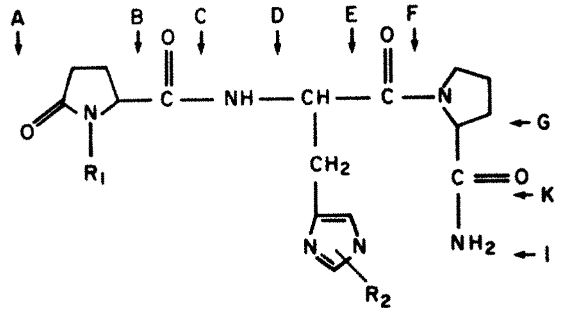


Fig. 4. Low resolution mass spectra of methylated ovine TRF (*a*) and synthetic PCA-His-Pro-NH_2 (*b*).

Table 2. CHARACTERISTICS OF PRINCIPAL IONS OBTAINED BY FRAGMENTATION OF OVINE TRF (METHYLATED DERIVATIVE) IN HIGH RESOLUTION MASS SPECTROSCOPY



Fragmentation points	Measured mass	Error mMU	Elemental composition
A→B	84·0463	+1·5	C_4H_6NO
A→B, $R_1 = CH_3$	98·0576	−3·0	C_5H_8NO
D→E	94·0535	+0·4	$C_5H_6N_2$
D→E, $R_2 = CH_3$	108·0673	−1·4	$C_6H_8N_2$
F→K, minus 4H	93·0211	−0·4	C_5H_3NO
F→I, minus 3H	110·0480	0·0	$C_5H_6N_2O$
G→I	44·0120	−1·6	CH_2NO
A→E, R_1 or $R_2 = CH_3$, minus H or C→K or D→I, minus H	234·1112	−0·4	$C_{11}H_{14}N_4O_2$
C→I, minus 2H	248·1147	0·0	$C_{11}H_{14}N_5O_2$
A→E, $R_1 = R_2 = CH_3$ or D→I, $R_2 = CH_3$ or C→K, $R_2 = CH_3$, minus H	249·1327	−2·4	$C_{12}H_{17}N_4O_2$

$R_1 = R_2 = H$, except when indicated otherwise in the table. Arrows and letters A–K indicate fragmentation points in mass spectroscopy. In non-derivatized ovine TRF, $R_1 = R_2 = H$.

Table 3. AMMONIA AND HISTIDINE CONTENTS IN 6 M HCl HYDROLYSATES OF ISOLATED OVINE TRF AND SYNTHETIC PEPTIDES

	No. of determinations	nmoles His	nmoles NH_3*	NH_3/His
Synthetic PCA-His-Pro-NH_2	6	38·4 ± 4·6†	40·3 ± 6·1	1·07
Synthetic PCA-His-Pro-OH	5	39·1 ± 3·3	0·4 ± 3·8	0·01
Ovine TRF	2	20·5 ± 2·1	22·1 ± 1·0	1·08
Ovine TRF unhydrolysed	1	—	−2·0	—

* Corrected for exogenous NH_3 in blank hydrolysed with each determination; average blank = 15·0 ± 5·7 nmoles NH_3 for seven determinations.

† Standard error.

activity of 44,000 ± 1,100 U/mg; the two figures for the respective specific activities are not statistically different. Natural ovine TRF as well as synthetic PCA-His-Pro-NH_2 are regularly active ($P \leq 0{\cdot}05$) in stimulating release of TSH *in vivo* (mouse TRF assay) in doses of 5 ng/animal, as a single intravenous injection; occasionally animals give a statistically significant response to a dose of 1 ng. Synthetic PCA-His-Pro-HN_2 stimulates release of TSH when administered orally to the assay animals in a dose of $\leq 1{\cdot}0$ μg (ref. 11). *In vitro*, hypothalamic ovine TRF or synthetic PCA-His-Pro-NH_2 stimulate release of TSH ($P \leq 0{\cdot}05$ in complete four-point assays with factorial analysis) at doses of ≥ 50 pg/ml. incubation fluid; this corresponds to about 1×10^{-10} mole TRF/ml./mg pituitary tissue. As is the case with natural TRF[12], pretreatment of the *in vitro* incubated pituitary with tri-iodotyronine (T_3) inhibits the response to the synthetic tripeptide amide (Table 4). The *in vivo* pituitary response as a function of time measured by plasma TSH concentrations, following intravenous injection of synthetic PCA-His-Pro-NH_2 (Table 5), is not statistically different from that observed for natural ovine TRF[13]. Pretreatment of the assay animals with a large dose of T_3 (0·25 μg) 2 h before injection of three different doses of synthetic tripeptide amide shows (Table 6) the same type of "competitive inhibition" as reported earlier for natural ovine TRF[14].

Table 4. BIOLOGICAL ACTIVITY OF PCA-His-Pro-NH_2 (X): *in vitro* RELEASE OF TSH; INHIBITION BY T_4

	mU TSH released/ pituitary/h	Potency ratio (95 per cent confidence limits)
C: Saline	1·1	
T: 5×10^{-11} g (X)/ml.	3·4	T/C = 2·5 (1·8–3·7)
C: Saline	0·8	
T: 5×10^{-10} g (X)/ml.	5·2	T/C = 7·2 (5·2–11·1)
C: 5×10^{-10} g (X)/ml.	5·1	
T: 5 μg T_4 pretreatment; 5×10^{-10} g (X)/ml.	2·8	T/C = 0·51 (0·35–0·72)

In vitro incubation of rat pituitary tissues as described in ref. 12; mU/TSH released/h calculated for all fluids in a single multiple four-point assay against TSH international standard. Potency ratio with confidence limits calculated by factorial analysis; see ref 23 for a complete mathematical description of these calculations for TSH/TRF assay. C, Control hemi-pituitaries; T, treated hemi-pituitaries.

Table 5. ACTIVITY OF PCA-His-Pro-NH_2 (X): ELEVATION OF PLASMA TSH IN NORMAL RATS

Treatment	Time (min)	μU TSH/ml. plasma (95 per cent confidence limits)	P
Saline	0	159 (82–248)	
Saline	+3	153 (76–243)	—
Saline	+10	115 (47–189)	—
Saline	+30	145 (71–226)	—
15×10^{-11} g (X)	0	131 (60–205)	
15×10^{-11} g (X)	+3	585 (360–1,570)	*
15×10^{-11} g (X)	+10	879 (500–3,170)	*
15×10^{-11} g (X)	+30	370 (238–736)	*

Plasma samples are aliquots from pools of six rats each receiving treatments as indicated, all animals anaesthetized with 'Nembutal' at time of injection (intrajugular); all injection volumes, 1·0 ml. *P*, Significance level by the multiple comparison test of Duncan; —, not different from control; * = 99 per cent; all calculations and statistical analyses as in ref. 23.

Table 6. ACTIVITY OF PCA-His-Pro-NH_2 (X): INTERACTION BETWEEN T_3 AND (X) ON THE RELEASE OF TSH *in vivo*

Treatment	N	TRF assay adj. $\log_{10}$ c.p.m. ± S.E.	P
Saline	3	3·1279 ± 0·0617	
5×10^{-12} g (X)	4	3·4657 ± 0·0534	*
2×10^{-11} g (X)	5	3·6132 ± 0·0493	*
8×10^{-11} g (X)	6	3·8218 ± 0·0437	*
T_3, saline	6	3·1244 ± 0·0436	
T_3, 5×10^{-12} g (X)	6	3·1065 ± 0·0437	—
T_3, 2×10^{-11} g (X)	7	3·1605 ± 0·0404	—
T_3, 8×10^{-11} g (X)	6	3·3022 ± 0·0437	—
T_3, 1×10^{-9} g (X)	6	3·4803 ± 0·0436	*

Experimental design and mathematical analysis as in ref. 14; *N* = number of assay animals/treatment; *P* as in Table 5; T_3: 0·25 μg intraperitoneal injection of tri-iodothyronine 2 h before TRF injection.

Synthetic PCA-His-Pro-NH_2, like natural ovine TRF[15], elicits a highly specific response in the release of TSH: PCA-His-Pro-NH_2 does not stimulate secretion of adrenocorticotropin or luteinizing hormone according to measurements performed concomitantly with TSH in the fluids of *in vitro* pituitary incubations; similarly, PCA-His-Pro-NH_2 does not stimulate secretion of luteinizing hormone *in vivo* in the rat (plasma levels measured by solid-phase radioimmunoassay) whereas it does stimulate secretion of TSH. Pure TRF has no oxytocic activity (rat uterus *in vitro*), no smooth muscle stimulating activity (guinea-pig ileum *in vitro*), no vasopressor activity (blood pressure recording of the pithed rat), no MSH activity (*in vitro* assay of Shizume *et al.*[16]). The biological activity of synthetic PCA-His-Pro-NH_2 is destroyed by incubation with plasma like that of hypothalamic ovine TRF (ref. 17 and W. V., R. B., T. F. D. and R. G., manuscript submitted). Furthermore, incubation of natural ovine TRF with a microbial pyrrolidone-carboxylyl peptidase (R. E. Fellows and A. Mudge, manuscript in preparation) destroys the biological activity; at the same time it liberates a ninhydrin-positive peptide which, as the free peptide or its dansyl-derivative, has chromatographic mobilities similar to those of a fragment—presumably His-Pro-NH_2—obtained by incubation of synthetic PCA-His-Pro-NH_2 in the same conditions. These results also favour the presence of an N-terminal PCA- in isolated ovine TRF. Plasma inactivation of TRF by a PCA-peptidase is an obvious possibility.

Conclusions in the Context of Other Work

Results from low and high resolution mass spectrometry, NMR and infrared spectrometry, thin-layer chromatographic data, amino-acid analyses and enzymatic degradation all favour the structure 2-pyrrolidone-5-carboxylyl-histidyl-proline amide (Fig. 6) for the ovine hypothalamic hormone TRF isolated in this laboratory. L-2-Pyrrolidone-5-carboxylyl-L-histidyl-L-proline amide prepared by synthesis has physical characteristics indistinguishable from those of natural ovine TRF; it also has similar biological activity.

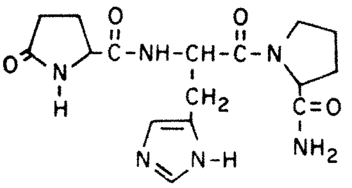


Fig. 6. Molecular structure of isolated hypothalamic ovine TRF.

Recently, Folkers *et al.*[18] and Bowers *et al.* (paper presented at a meeting of the American Thyroid Association, Chicago, November 1969) have confirmed that mixtures of synthetic peptides obtained by ammonolysis of the esterified derivatives of the sequence Glu-His-Pro have considerable TRF activity, in agreement with our earlier reports. Their statement that the synthetic peptides are not active orally is probably best explained by the fact that they administered doses of the materials which were too low. More recently, the same group[19] has reported on the similarity in properties of a preparation of porcine TRF[20] and a synthetic preparation "presumably (pyro)-Glu-His-Pro-NH_2" in a variety of thin-layer chromatographic systems and bioassays; characterization of the synthetic material was claimed on the basis of "its hydrolysis to the three amino-acids", its NMR spectrum and thin-layer chromatographic mobilities. Although other possibilities were considered, the authors concluded that the structure PCA-His-Pro-NH_2 was in agreement with the known properties of porcine TRF; these reports are reviewed in ref. 21. If the conclusions of Bøler *et al.*[19] are correct, then the structures of hypothalamic TRFs from two different species would be identical.

Our results, concerning the ovine TRF as it was isolated in our laboratory, do not exclude the possibility that the native molecule might exist as Gln-His-Pro-NH_2, the N-terminal glutamine having been cyclized to PCA-during the extraction procedure; there are precedents for this in the chemistry of the eledoisins[22]. The possible activity of synthetic Gln-His-Pro-NH_2 has not yet been studied.

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Defective Viral Particles and Viral Disease Processes

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Preparations of many different types of viruses contain defective particles which consist of viral structural proteins and a part of the viral genome. Such particles are capable of specifically interfering with the growth of homologous, standard virus and may play a major part in the evolution of viral diseases.

Biologically active defective viral particles have been described with increasing frequency in animal virus systems, suggesting that they may be more common than had been suspected. We propose that such defective particles have a role in determining the evolution of viral diseases. Many host defence mechanisms are known which combat viral infections[1,2]. These involve antiviral antibodies and lack of attachment sites on host cells. General factors such as fever, localized acidity, heredity and interferon have also been implicated but, except for some tentative suggestions[3-5], the defective virus has been overlooked as a possible important determinant in the outcome of natural viral infections.

To support our hypothesis that defective viral particles play a part in the course of viral infections, the present knowledge of defective particles will be summarized. Some *in vitro* studies will be presented which relate to known disease processes, and an explanation at the molecular level will be offered for the appearance of defective particles in viral preparations and for the biological activity of defective particles.

The defective particles which are pertinent here have the following properties: (1) they contain normal viral structural protein; (2) they contain a part of the viral genome; (3) they can reproduce in the presence of helper virus; (4) they interfere specifically with the intracellular replication of non-defective homologous virus. When defective particles are added to infected cells, the yield of non-defective virus is reduced. This interference can occur in the absence of detectable interferon and is dependent on the nucleic acid contained in defective particles. As will be seen, this specific interference by defective particles may be an important mechanism in virus–host interactions.

Because defective virus with the properties mentioned above occur in many and possibly all animal virus systems, we propose to call them DI particles (DI = defective interfering). The non-defective homologous virions which are capable of independent replication are called standard virus.

Animal Viruses which form DI Particles

Von Magnus[6] first described "incomplete" or DI particles, produced after inoculation of fertilized eggs with high multiplicities of influenza virus. The DI particles are antigenically identical to standard virions[7,8] but are lacking the largest of the RNA species found in standard virions and contain small, unidentified pieces of RNA[9].

The synthesis of similar DI particles by cells or animal tissues on infection with high multiplicities or "undiluted passage virus" has now been reported for Rift Valley fever virus[10], vesicular stomatitis virus[11], fowl plague virus[12], simian virus 40 (ref. 13), polyoma virus[14], lymphocytic choriomeningitis virus[15], Sendai virus[16], simian virus 5 (personal communication from P. W. Choppin), and poliovirus (unpublished observations). Of these viruses, vesicular stomatitis DI particles are the best characterized in respect of biological and physical properties because they differ in length and are readily separated by rate zonal centrifugation[17-22].

Defective Particles in Viral Disease: A Model

From our studies on vesicular stomatitis virus and from the published data on influenza, we have composed a diagrammatic representation of the virus–host interaction in conditions where DI particles are produced (Fig. 1). Initially, one standard particle infects a cell and produces more standard virions which then infect other cells. Very occasionally, a DI particle arises in a cell infected with standard virions. Once the DI particle appears in a viral preparation, it begins to replicate together with standard virions and eventually DI particles are the predominant component in the viral progeny. As more DI particles are produced, less standard virions are present. Because their ability to replicate is dependent on standard virions, the concentration of DI particles will decline as the infection spreads until a few DI and standard virions are left. At this stage specific antibodies or other factors can neutralize the remaining virions. This course of events, as depicted in result II, Fig. 1, may represent acute self-limiting viral disease.

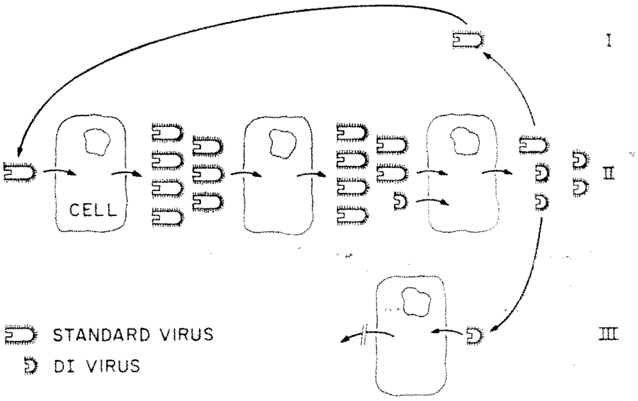

Fig. 1. A model for the role of DI particles in the course of viral infections.

In the absence of antibodies and in the presence of only a few viral particles, cells will be infected by only single DI or standard virions. Cells infected with only standard virions will produce a new crop of standard virions (result I, Fig. 1) while cells infected with only DI particles will produce no yield (result III, Fig. 1). The consequence will be an overall increase of standard virions and a new cycle of infection. Repeating cycles of infection could continue indefinitely, producing recurrent bouts of acute illness or viraemia, but synthesis of standard and DI particles may be in such balance that only a very few virions are synthesized and the infection would remain in a subacute or latent form until the balance is upset.

Because the model system seems to fit descriptions of viral disease processes, let us look at some of the data on viral infections to see if there is support for the existence of DI particles during natural infections.

Acute and Persistent Infections

Although many viruses known to cause acute illnesses produce DI particles in laboratory conditions, DI particles have not been isolated from naturally occurring infections in animals. Nevertheless they are not artefacts of cells in tissue culture. They are produced in mouse brains[10] and in chick embryos[6,16,23]. The ability of DI particles to prolong survival has been documented for influenza virus[24] and Rift Valley fever virus[10]. The present understanding of DI particles enables us now to study them in relation to the evolution of acute viral diseases.

One of the best studied persistent viral diseases is lymphocytic choriomeningitis (LCM). In adult mice, infection with LCM virus is fatal, but young mice become immunologically tolerant, and the virus persists throughout their lifetime[25,26]. Interferon is not found in the infected animal nor does LCM virus induce the synthesis of interferon in cell cultures[27]. Lehmann-Grube *et al.*[15] established a persistent culture of L-cells by inoculating them with a high multiplicity of LCM virus. Only DI particles were produced and the cells exhibited intracellular interference with superinfecting standard virus, suggesting that the DI particle and its specific interference might maintain the persistent infection in adult, tolerant mice.

Defective particles of measles virus[28] may be an interfering agent in conditions where few standard particles are recovered and large amounts of viral nucleoprotein tubules remain associated with infected cells[29,30]. The situation seems to be similar to that in cells infected with fowl plague virus, in which, in conditions of interference by DI particles, viral ribonucleoprotein accumulates in the cells[12]. Measles virus has long been suspected of being related to the causative agent of a disease of the central nervous system known as subacute sclerosing panencephalitis (SSPE). Recent isolations of the virus from a brain biopsy suggest that measles virus can persist in its host and cause the disease of SSPE[31-33]. This may be the result of interference by DI particles, accounting for the slow progression of the disease, as well as for the difficulties encountered in isolating standard measles virus from infected brain tissue.

Besides interfering with the growth of standard virus, DI particles often have other properties which are consistent with their content of viral structural proteins and part of the viral genome. For example, vesicular stomatitis DI particles inhibit host RNA synthesis[34], fowl plague DI particles produce fluorescent antigen[12], and DI particles of simian virus 40 produce T antigen[35]. Thus, depending on the particular virus system, DI particles may show a variety of particle–host interactions in the absence of standard virions.

Control by the Host Cell

A number of factors seem to affect the synthesis of DI particles but none have been studied extensively. Some *in vitro* experiments show that the species of host cell greatly affects synthesis of DI particles. Madin-Darby bovine kidney cells can be infected at high multiplicities with influenza virus without marked interference, whereas chick cells of the chorioallantoic membrane readily produce influenza DI particles[23]. When vesicular stomatitis virus is grown in identical medium and culture conditions, but in two different cell lines, the bovine kidney cells or Chinese hamster ovary cells, different ratios of the two types of virus particles are made depending on the species of host cells. Table 1 shows the production of standard VSV particles after successive undiluted passages. Interference readily occurs in Chinese hamster ovary cells where two successive undiluted passages reduce the standard virus to 0·1 per cent of the original titre. Moreover, the harvest of DI particles of VSV is highest after the first undiluted passage; successive passages also result in reduced synthesis of DI particles[36]. By contrast, Madin-Darby bovine kidney cells produce standard VSV at about 10 per cent of the original titre through five successive undiluted passages.

These results suggest that host cells exert control over the synthesis of DI particles. The permissiveness of certain cells or tissues to production of DI particles may account for the localization of certain viral diseases as well as for the variation in virulence of a given virus in different hosts.

It is noteworthy that by the fourth successive undiluted passage of VSV in Chinese hamster ovary cells, standard virus undergoes a burst of synthesis as predicted by the model (result II, Fig. 1). Such cyclic activity has also been observed in a cell culture persistently infected by another plaque variant of VSV, although the production of DI particles was not measured[37]. Even with one of the most virulent viruses, therefore, persistently infected cell cultures can be maintained in the absence of antibody or significant amounts of interferon.

Table 1

Undiluted passage*	Plaque forming units per ml. Chinese hamster ovary cells	Plaque forming units per ml. Madin-Darby bovine kidney cells
1	$7{\cdot}55 \times 10^8$	$4{\cdot}68 \times 10^8$
2	$3{\cdot}02 \times 10^6$	$1{\cdot}25 \times 10^8$
3	$4{\cdot}40 \times 10^4$	$1{\cdot}00 \times 10^8$
4	$3{\cdot}38 \times 10^7$	$3{\cdot}95 \times 10^8$
5	—	$4{\cdot}60 \times 10^8$

* A VSV (large plaque, Indiana serotype) preparation at 2×10^9 plaque forming units/ml. was used at an input multiplicity of 100 to infect either Chinese hamster ovary or Madin-Darby bovine kidney cells (a gift from Dr P. W. Choppin) grown to confluence in a 100 mm Falcon plastic Petri plate in Joklik's modified minimal essential medium (Grand Island Biological) with added non-essential amino-acids and 7 per cent foetal calf serum. After incubation at 37° C in a 5 per cent CO_2, humidified incubator for 16–18 h, the medium was collected. Successive undiluted passages were made by infecting similar cell monolayers with 0·5 ml. of the previous yield followed by the same conditions of incubation. Standard VSV grown in Chinese hamster ovary or Madin-Darby bovine kidney cells were assayed for plaque formation on Chinese hamster ovary monolayers as described previously[38].

The age of the animal and temperature of incubation may also affect the production of DI particles. Experiments with chick embryos show that VSV grows better at 34° C than at 39° C (ref. 38) and that 7 day old embryos support the growth of standard VSV better than 10 day old embryos. Although DI particles were unknown at the time of these studies, they may have played a part in the prolonged survival of chick embryos infected with VSV and incubated at the higher temperature.

Many other factors, such as conditions prevailing in the inflammatory zone, heredity, and hormonal agents may also affect synthesis of DI particles. Undoubtedly, observations concerning DI particles will become more common as the large number of factors influencing their production become understood.

The Mechanism of Interference

Production of DI particles is a two step process. The first is the initial formation of a single DI particle from a preparation containing only standard virus. The second is the replication of the DI particle at the expense of standard virus and its ultimate domination of the virus yield. The latter stage represents the phenomenon of interference.

Because DI particles arise from clonally purified standard particles[14,35,39,40], the first stage must be the initial synthesis of a small piece of nucleic acid capable of combining with viral structural proteins to form a defective particle. The frequency of formation and the size of such a small piece of nucleic acid must necessarily depend on the mechanism of nucleic acid replication of the different viruses and might be viewed as the production of a deletion mutant. For instance, influenza virus which has its genome in several small pieces forms DI particles with greater frequency than poliovirus which contains a single piece of RNA. Also, viruses which normally synthesize smaller pieces of nucleic acid for purposes other than incorporation into progeny virions might be expected to have a relatively high frequency of formation of DI particles. An understanding of the replication of nucleic acids of different viruses, therefore, would shed light on the frequencies of occurrence of DI particles for different viruses as well as for different strains of the same virus. Whether the initial appearance of DI particles is also dependent on a host cell factor is at present unknown.

Interference is usually detected when cells are infected with high multiplicities of unpurified virus or when purified DI particles are added to cells infected at low multiplicities with standard virus. These conditions favour the co-infection of cells by DI and standard virions. With VSV in tissue culture cells, complete interference is correlated with cessation of all viral RNA synthesis; whereas, during partial interference, there seems to be a competition between the synthesis of small RNA and large RNA[36]. Interference by DI particles may be analogous to a phenomenon which occurs during the *in vitro* synthesis of bacteriophage Qβ RNA. Small Qβ RNA fragments compete successfully with the large complete Qβ RNA for replicase and ultimately dominate the reaction (ref. 41 and personal communication from L. E. Orgel).

We conclude that DI particles may be important determinants of the course of acute, self-limiting viral infections and of persistent, slowly progressing viral diseases. In addition, many host reactions may alter the production of DI particles and thus influence the outcome of viral infections. The test of these ideas lies in future experiments on the details of the course of viral infections.

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Magnetic Anomalies and Seafloor Spreading Rates in the Northern South Atlantic

by

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A geomagnetic profile across the northern South Atlantic yields spreading rates for the last 70 m.y. which vary from 1·6 to 2·0 cm/year. There is evidence for three regional discontinuities in the spreading history of the South Atlantic.

FOLLOWING the development of the Vine–Matthews hypothesis of seafloor spreading[1], spreading rates have been determined for the North Atlantic between the Reykjanes Ridge and 11° N latitude[2,3] and for the extreme southern Atlantic[4]. These rates enabled LePichon[5] to demonstrate that the American and European–African blocks rotated around a pole located, during the latest phase, near south-eastern Greenland. Supporting data from the northern South Atlantic have so far been lacking.

On the CIRCE Expedition of the Scripps Institution of Oceanography in November 1968, we obtained a geomagnetic profile from Luanda, Angola, to Fortaleza, Brazil (Fig. 1). The total field magnetic readings were digitized and the International Geomagnetic Reference Field was removed by computer[6]. In the centre of the ocean, the anomalies are predominantly negative with respect to the reference field; near the Brazilian and African continental margins, they become positive. The profile crosses the east flank of the mid-Atlantic Ridge between 7° and 8° S latitude, approximately perpendicular to the ridge axis. Just beyond anomaly 4 on the west flank, it is interrupted by a northward leg crossing the Ascension fracture zone, and the principal west flank crossing starts to the west of the axis in the block north of the fracture zone. Here, the track veers approximately 70° W of the ridge axis.

The profile is relatively close to the magnetic equator, which lies approximately 20° N. Nevertheless, by comparison with North and South Pacific standard profiles, and profiles published for the South Atlantic[4], many anomalies could be identified with a reasonable degree of certainty, especially numbers 5, 6, 12, 13, 18, 20 and 21 (Fig. 1). The identity of intermediate anomalies was then established. On the east flank, anomalies up to 32 could be recognized; on the west flank, the pattern extends out to anomaly 30. Detailed surveys in four locations on the east flank confirm that the anomalies veer slightly west of north, and that offsets do not exceed 10 km, thus providing a confidence limit for the distance estimates. There is no evidence that fracture zones other than the Ascension fracture zone were encountered.

Ages for the numbered anomalies have been proposed by Heirtzler *et al.*[7]. Recent data from the Atlantic operations of the drilling vessel Glomar Challenger[8] agree with this time scale.

A plot of the assumed ages of the east and west flank anomalies against distance from the ridge axis is given in Fig. 2. For the west flank, the distances were corrected by projecting the anomaly peaks on a line perpendicular to the ridge axis; for the east flank, such a correction is not necessary. Anomalies 1–4 of the west flank are on a direct extension of the east flank profile; for anomaly 6

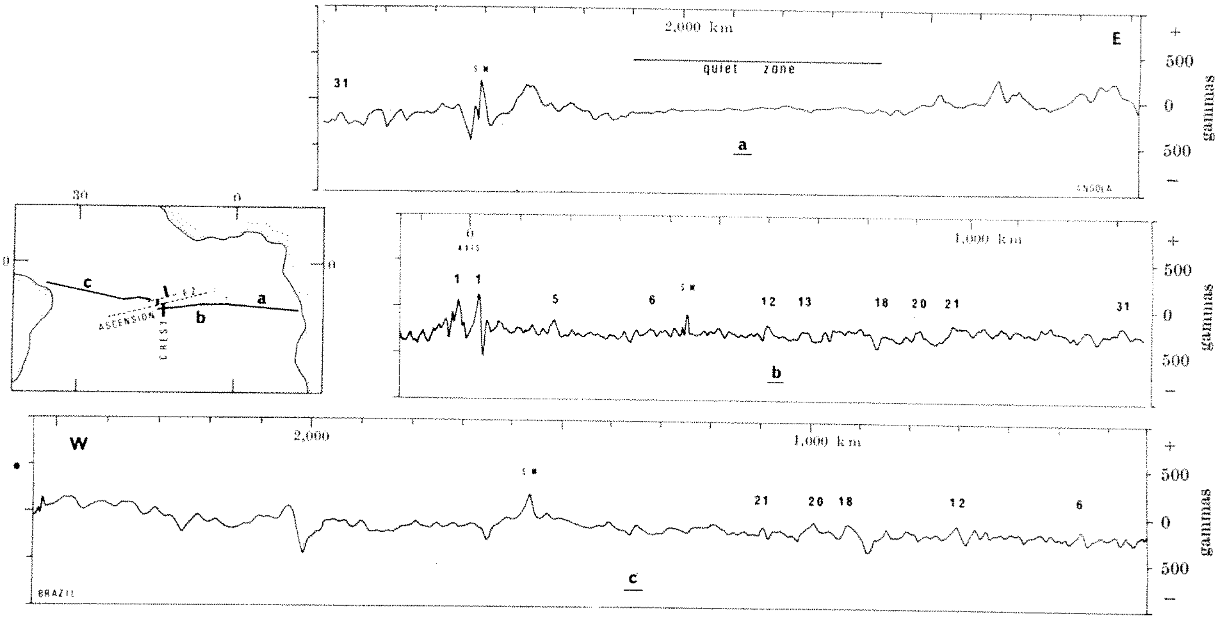

Fig. 1. Geomagnetic anomalies in the northern South Atlantic along CIRCE track shown in inset. Numbered anomalies correspond to classification of Heirtzler *et al.*[7]. For details of correlation, compare Figs. 3, 4 and 5 in Dickson *et al.*[4]. Sea-mounts affecting anomaly pattern are labelled SM.

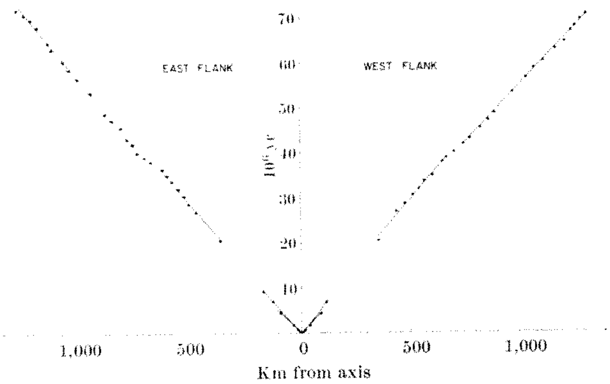

Fig. 2. Distance from ridge axis of numbered anomalies on CIRCE profile plotted against time scale of Heirtzler *et al.*[7]. Axial position for anomaly 6 and beyond on west flank determined by extrapolation backward, using spreading rate for anomalies 1–4. Distances for west flank projected on line perpendicular to ridge axis. Regression lines fitted by least squares (see Table 1).

and beyond, located in the block north of the Ascension fracture zone, the position of the axis was determined by extrapolation from anomaly 6, using the spreading rate obtained from anomaly 1–4. The position of the axis so obtained is in accordance with earthquake epicentre locations[10], and shows a left lateral offset of approximately 80 km across the Ascension fracture zone.

On each flank, the points fall close to several straight line segments. The least squares fit of the regression lines shows very high correlation coefficients, small standard errors for the spreading rates, and high levels of significance for most differences between spreading rates—both for successive line segments and for opposite pairs on east and west flanks (Table 1). The rates for the most recent phase of spreading are in good agreement with the pole of rotation postulated by LePichon[5].

Table 1. SPREADING RATES IN THE SOUTH ATLANTIC OCEAN BETWEEN 6° AND 8° S LATITUDE

Anomaly number	n	r	West flank spreading rate	Standard error	n	r	East flank spreading rate	Standard error
0– 3	4	0·99	1·89 cm/yr	0·04	4	0·99	2·16 cm/yr	0·24
0– 5	5	0·99	1·60	0·14	6	0·99	1·82	0·10
7–12	6	0·99	1·80	0·06	6	0·99	1·78	0·06
14–32	17	0·99	1·98	0·02	18	0·99	1·70	0·02

n : number of anomalies in series.
r : least squares correlation coefficient.

Spreading rate differences between successive series on each flank and between opposite series on east and west flank are significant at the 95 per cent level or better (t test), except for the 0–5 and 7–12 series on the east flank which are significantly different only at the 90 per cent level, and 7–12 series on east and west flank which are not significantly different at the 75 per cent level.

Spreading rates between the axis and anomaly 3 are approximately 2 cm/yr with the east side spreading slightly, but significantly, faster. Between anomalies 3 and 4 there is a discontinuity. A discontinuity at this point has also been observed in the North Atlantic[2,3,11,12] and the North Pacific[1]. Another discontinuity between anomalies 5 and 7 is characterized on the east flank by an offset and a change in spreading rate. On the west flank, this discontinuity is not well established: the wide spacing of anomalies in this interval, the absence of anomaly 5, and the break in the record render the data for this section inconclusive. Ewing and Ewing, and Schneider and Vogt[13,14], have argued that there was a major discontinuity approximately 10 m.y. ago, either as a period of quiescence or as a marked reduction of the spreading rate, and LePichon[5] has included it in his reconstruction of the spreading history of the Atlantic. Our data modestly support these interpretations.

Between anomalies 7 and 12, a uniform spreading rate is obtained on both sides of the crest, followed by a brief but clear discontinuity approximately 35–38 m.y. ago. This discontinuity is marked by a temporary sharp decrease in the spreading rate. As far as we are aware, it has not been reported from elsewhere. It is followed by another and prolonged period of uniform spreading extending to the last observed anomalies. During this period, the spreading rate on the east flank is significantly lower than the rate on the west flank. The opposite would be expected because, according to LePichon's reconstruction for this period, the east flank here is at a greater distance from the rotational pole than the west flank. LePichon also showed that a considerable reorientation of the spreading direction has occurred for this phase. Projection of the CIRCE spreading rates on appropriate perpendiculars to the axis only increases the asymmetry. This asymmetry in spreading rate could account for the asymmetry of the Atlantic basin at this latitude. The rates are in reasonable agreement with the regional rate postulated by LePichon for the situation prevailing at the time of anomaly 31.

To compare the breaks observed in our profiles with other data from the South Atlantic, we have plotted the anomalies of Dickson *et al.*[4] against their assumed ages (Fig. 3), together with the CIRCE data. The profiles show, without exception, the discontinuity near anomaly 3, and in all cases except *V*-22 (where the upper bound is not clear), also the break near anomaly 6. The break between anomalies 12 and 13 or 14 is also well defined except in *C*-8. The data have been taken from figures in Dickson *et al.*[4] and are not highly accurate; nevertheless, the possible error of 10–20 km does not seriously affect the trends.

Fig. 3 shows that, relative to an arbitrary time scale, there have been several distinct and widespread discontinuities in the spreading history of the southern mid-Atlantic ridge. In the plate tectonic concept[5], such discontinuities can be explained either by an overall change

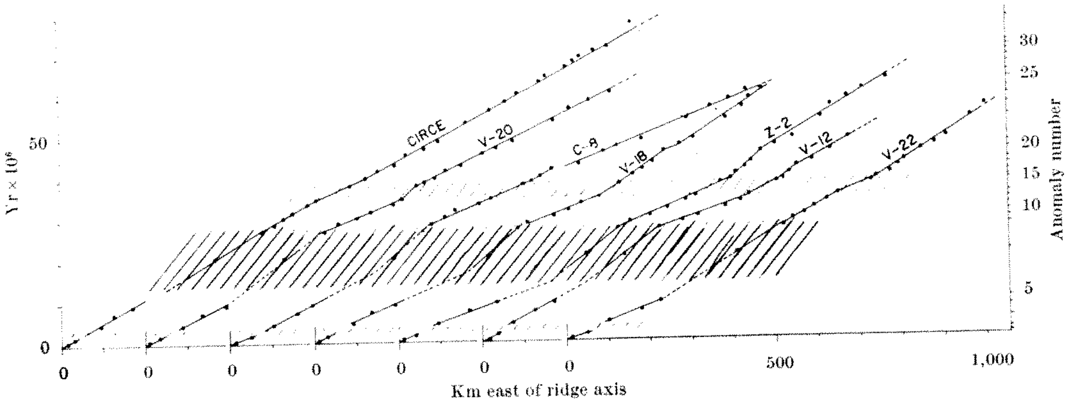

Fig. 3. Distances to ridge axis of numbered anomalies on east flank of mid-Atlantic ridge in the Southern Atlantic Ocean, plotted against geomagnetic time scale of Heirtzler *et al.*[7]. Data from Dickson *et al.*[4]. Shaded zones indicate regional discontinuities.

in the rate of production of new crust, or by a shift in the pole of rotation of the plates. A regional map of the anomalies would reveal a change in the pole of rotation as a reorientation of the trends of the anomalies. A change in spreading rate can only be established by reference to an absolute time scale calibrated by radiometrically or palaeontologically dated samples. Such a time scale may soon be possible.

The east flank profile shows several additional anomalies above 32, although the record here is disturbed by the presence of two sea-mounts. Beyond that area, there is a magnetically quiet zone, 400 km wide. Extrapolation of spreading rates suggests that the western edge of this quiet zone is about 100 m.y. old. On the west flank, no quiet zone is found; pronounced anomalies continue to the margin of the continent. Heirtzler and Hayes[15] described quiet magnetic zones flanking the mid-Atlantic ridge in the North Atlantic and they have suggested that these are produced by continued spreading during a period devoid of magnetic reversals. Drake and others[16], on the other hand, considered the quiet zones of the North Atlantic to be part of the floor of a proto-Atlantic Ocean which predates the onset of the current period of continental drift.

The absence of a quiet zone on the west flank of the CIRCE profile argues against the assumption that it is caused either by the process postulated by Heirtzler or by the effect of the low magnetic latitude. Drake's hypothesis is obviously attractive, but because the fit of the South Atlantic margins is excellent, new difficulties would arise from fitting the Brazilian margin against the westward limit of the quiet zone in the eastern basin. Dickson *et al.*[4] have noted the presence of a quiet zone in the Rio Grande basin, and show anomaly profiles with a quiet zone in the Cape basin, but there are insufficient data as yet to evaluate its regional distribution. The absence of a quiet zone in the western part of the northern South Atlantic indicates a very asymmetric distribution of spreading rates for the earliest spreading phase.

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How Thick is the Lithosphere?

by
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A rapid decrease in shear velocity in the suboceanic mantle is used to infer the thickness of the lithosphere. It is proposed that new and highly precise group velocity data constrain the solutions and imply a thickness near 70 km.

THE hypotheses of seafloor spreading and plate tectonics have received much attention lately because they explain in a consistent manner the pattern of magnetic anomalies on the seafloor, the distribution of earthquake belts and the thickness and ages of deep-sea sediments. According to these hypotheses the lithosphere (composed of the crust and the rigid cap of the upper mantle) is divided into a number of rigid plates in constant motion. The plates are created along midocean ridges and are destroyed in the process of sinking back into the mantle in the vicinity of deep-sea trenches. The lithosphere is underlain by the asthenosphere—a weak zone coincident with the seismic low velocity layer, and probably the source region of basaltic magma.

This article is concerned with the problem of determining the thickness of the suboceanic lithosphere. Thickness is an important parameter which enters discussions of the mechanism of plate tectonics and the thermal regime in the lithosphere.

The thickness of the lithosphere may be estimated in several different ways. For example, seismic focal depths under Hawaii presumably are coincident with the zone at the base of the lithosphere where magma enters the fissure system which ultimately leads to the surface. A value of 60 km was found[1]. Seismic focal depths at midocean ridges extend to 65 km, this limiting depth presumably marking the base of the lithosphere[2]. The most direct indication of the thickness of the lithosphere is the shear velocity distribution with depth. A reversal in shear velocity in the suboceanic mantle now seems to be uniquely established although the details of the velocity–depth curve are still uncertain. Presumably the high velocity lid to the low velocity zone coincides with the lithosphere and the base of the lithosphere is marked by a rapid decrease in shear velocity. This method has been used by several investigators and values ranging from 40 to 70 km have been reported. Unfortunately these determinations are subject to the well known lack of uniqueness of surface wave methods, especially when density and shear velocity are both allowed to vary.

In this article we use new and highly precise group velocity data to test models found by F. P. using a Monte Carlo procedure and reported in an earlier paper[3]. In the previous work randomly selected models were required to fit Love and Rayleigh wave phase velocities pertinent to oceanic paths, eigenperiod data, mass and moment of inertia of the Earth. In the new test, group velocities are calculated for each model and compared with the new group velocity values of H. K.[4] which are based on thirty sets of data for predominantly oceanic paths. These are more data than previously available, leading to good estimates of the errors. This is a stringent test because group velocity is associated with the derivative of phase velocity with respect to period and a slight difference in slope of phase velocity shows up more clearly in the group velocity curve.

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The results for Rayleigh waves are shown in Fig. 1. H. K.'s experimental group velocities together with error indications ($\pm\sigma$) are shown with group velocities calculated for various models. Also shown are the early data of Ewing and F. P.[5]. The Haddon–Bullen model HBI (ref. 6) fails to fit the data between 150 and 200 s. The Gutenberg–Bullen A model (ref. 7) fits the data somewhat better, but this model must be rejected because of its failure to fit the Love wave observations (not shown here). The well known models **8099** (ref. 8) and CIT 11A (ref. 9) do not fit the new Rayleigh wave group velocity data. All twenty-eight models of F. P. fit the Love wave group velocities but only seven fit the Rayleigh wave data within $\pm 2\sigma$. Two of these are shown in Fig. 1 (5·08, 9·71) together with two models which fail (6·21, 7·77).

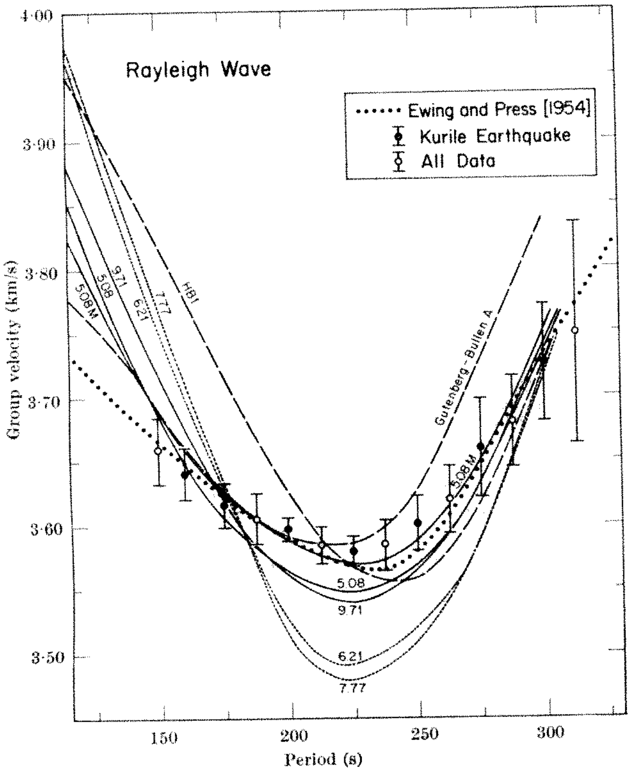

Fig. 1. Rayleigh wave group velocity data compared with several theoretical models; vertical bars indicate $\pm\sigma$.

The seven successful models are illustrated in Fig. 2. All show a large decrease in shear velocity beginning at 70 km. In the absence of an analytical method for establishing uniqueness with the data used, we interpret the narrow envelope formed by the models between 70 and 145 km as strong evidence that the data highly constrain the solutions. Numerical experiments were made in which the models were varied over smaller steps than with the Monte Carlo solutions. What seems to be required to find solutions which fit the group velocity data to $\pm 1\sigma$ is an almost abrupt velocity drop at a depth of 70 km as shown by model 5·08 M in Fig. 3. The fit of this model to the data is shown in Fig. 1. From these results we conclude that the base of the lithosphere is at a depth not much different from 70 km.

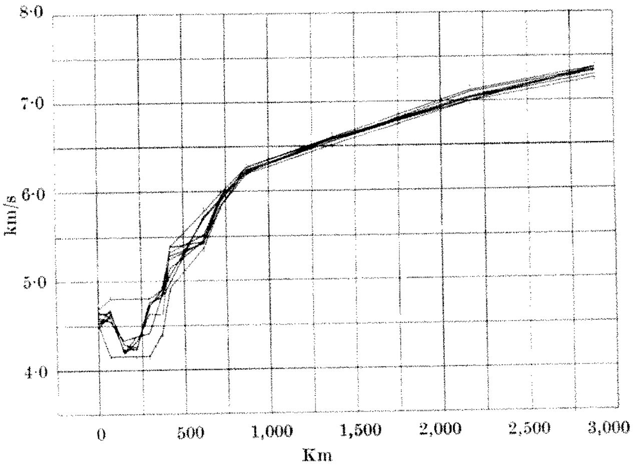

Fig. 2. Shear velocity distributions in the Earth's mantle which fit new group velocity data.

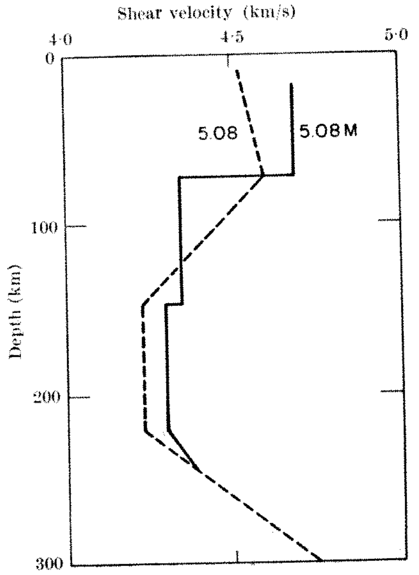

Fig. 3. Modification of model 5·08 to 5·08 M to obtain better fit to group velocity data.

Although several previous investigators (see ref. 10, for example) found similar results, we believe that our procedures remove the uncertainties which accompany surface wave studies. This is based on the agreement between models in which density and shear velocity were randomly selected and subjected to tests against data of known precision.

The sharp decrease in rigidity near 70 km supports the notion that the lower boundary of the lithosphere is determined by the solidus.

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LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Linear Polarization of Distant Quasars

MEASUREMENTS of the linear polarization of quasars at a wavelength of 49 cm (m_{49}) have already shown that this parameter is correlated with spectral index α (ref. 1). We have now observed a more complete sample of 93 quasars, all of which have measured redshifts. The correlation between m_{49} and α is confirmed, but a further correlation has been detected, namely between m_{49} and redshift z. The relation between m_{49} and z is shown in Fig. 1. The redshifts are taken from Burbidge and Burbidge[2], Schmidt[3] and Hiltner *et al.*[4]. The spectral indices represent the spectral slope at or near 49 cm. The separation between flat spectrum and steep spectrum quasars is especially marked for low redshifts ($z < 1{\cdot}25$). This separation does not exist for polarization measured at shorter wavelengths, and is therefore a depolarization effect, presumably by Faraday rotation within the source. Fig. 1 shows that the median value of m_{49} falls markedly as z increases for flat spectrum quasars. The median value of m_{49} is almost independent of z for steep spectrum quasars.

Both these trends with z are at variance with what one might expect on simple arguments. Measurements at a given observed wavelength correspond to emission at an emitted wavelength λ_{em} which progressively decreases with increasing z. For example, our observations correspond to $\lambda_{em} = 21$ cm for $z = 1{\cdot}3$ and $\lambda_{em} = 14$ cm for $z = 2{\cdot}5$. Because, on average, sources show an increase of polarization with decreasing wavelength, one would expect an increase in median m_{49} with z. This argument applies whether or not the redshift z is cosmological in nature.

Linear polarization has been measured in the wavelength range 6–21 cm (refs. 5–9) for 40 out of the 68 quasars with $z < 1{\cdot}25$. We have used these data to estimate the median polarization as a function of emitted wavelength, from which we may deduce the expected polarization as a function of observed wavelength for quasars of high z. The dashed lines in Fig. 1 show the results of this analysis, and indicate that quasars of both spectral types show less polarization at λ49 cm than expected. In each case the reduction is by a factor of about three.

A similar analysis for a shorter observed wavelength (λ18 cm) shows no effect, so the reduction we observe is not a difference of intrinsic polarization, but must be due to a depolarizing mechanism. In view of the similarity in reduction factor for steep spectrum and flat spectrum quasars, the depolarization is unlikely to be due to dilution by unpolarized components with abnormal spectra.

Faraday depolarization in the Milky Way does not produce a significant effect as the sources all lie at galactic latitudes $> 10°$. The effect of other, intervening, galaxies has been discussed by Wagoner[10] and others[11]. The probability that a galaxy lies on the line of sight to a quasar at $z = 2$ is about 10 per cent, depending on the size and space density of galaxies out to this distance. The depolarization we observe might be produced by Faraday rotation in randomly distributed objects, but only if their size or space density beyond $z = 1$ exceeds the local value for galaxies.

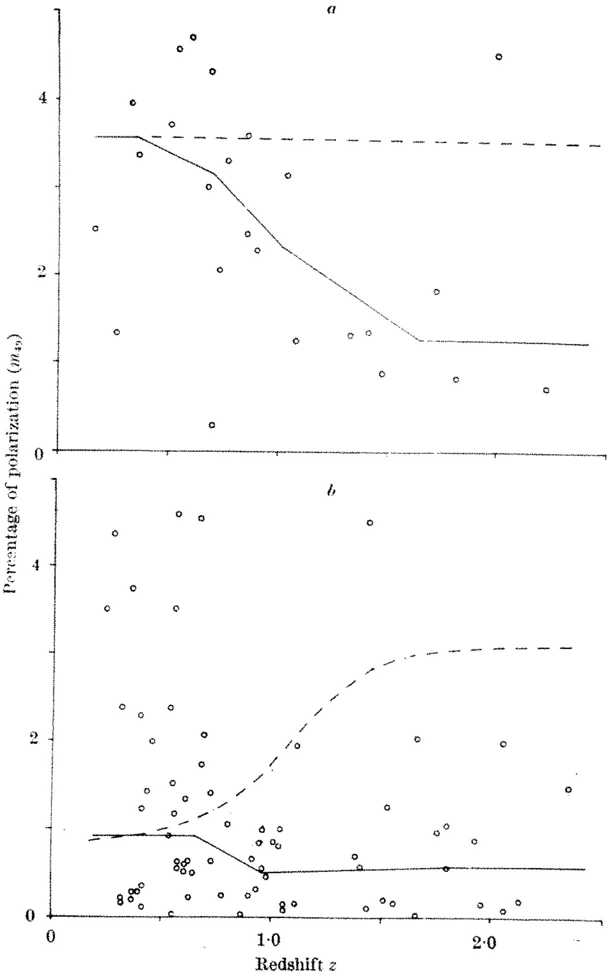

Fig. 1. The polarization (m_{49}) of (*a*) flat ($\alpha > -0{\cdot}5$) and (*b*) steep spectrum ($\alpha < -0{\cdot}5$) quasars. The solid lines represent the median lines. The dashed lines show the expected trends (see text).

Whether the intervening objects are randomly distributed, or are physically associated with the sources, they should show other absorption effects. Table 1 compares the linear polarization of high redshift quasars with and without redshifted absorption lines[2]. Those with absorption lines show significant depolarization compared with the remainder. Though the size of the sample is small, we believe that the depolarization is due to Faraday rotation in the same regions as are responsible for the absorption lines.

Table 1. QUASARS WITH REDSHIFT $z > 1{\cdot}25$

	Number	$\overline{m}_{49}$
Absorption lines	7	$0{\cdot}56 \pm 0{\cdot}21$
No absorption	18	$1{\cdot}38 \pm 0{\cdot}29$
Difference		$0{\cdot}82 \pm 0{\cdot}36$

Faraday depolarization requires that the rotation in the intervening cloud shall be at least 2 rad at the wavelength concerned. For $Z_{abs} = 2$ this wavelength is 16 cm, and hence the necessary rotation measure is ≥ 80 rad m^{-2}. The integral $\int N_e . B_{\|} . dl$ to achieve such a rotation measure is 10^{-4} cm^{-3} G pc. For comparison, a line of sight passing through a spiral galaxy suffers a rotation measure of about 30 rad m^{-2}.

This information from the Faraday rotation should be of great importance in helping to define the nature of the objects responsible for the absorption lines.

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Upper Limit on the Intensity of Sgr γ-1 in Soft X-rays

FRYE *et al.*[1] have reported evidence for a point source of γ-rays, Sgr γ-1, with an intensity $I_{\mathrm{Sgr}\,\gamma-1}(>50\ \mathrm{MeV}) = (3 \pm 1) \times 10^{-5}$ photons cm^{-2} s^{-1}. It was seen in two separate balloon flights in February 1969 at a significance level of about three standard deviations of the background rate. Frye *et al.* give the coordinates of the source as right ascension = 19 h 12 m ± 12 m and declination = $-35° \pm 2°$.

It is important to have confirmation of the existence of the source Sgr γ-1. Assuming the source is real, however, we can set an upper limit on its intensity in soft X-rays (1·5–6 keV) from an Aerobee rocket experiment flown on July 7, 1967, by Bradt *et al.*[2]. Two banks of proportional counters, with 50 μm beryllium windows, each of area 350 cm^2, were collimated to 2·1° × 20° (FWHM). The long directions of the fields of view crossed one another at 60° and the centre of the field of view moved along a path of almost constant declination of $-24°$ from right ascension 21 h 06 m to 18 h 12 m at a fast roll rate of 3·5° s^{-1}. Subsequently the detectors scanned the Sagittarius sources, Sco X-1 and M-87, at a slow roll rate of 0·3° s^{-1}. During the fast roll each bank of counters looked at the Sgr γ-1 source for 1·4 s. The altitude of the rocket varied between 109 and 120 km during the fast roll. The counting rate in the 1·5–6 keV energy region in each bank remained stable at 55 counts s^{-1} or ~0·16 counts cm^{-2} s^{-1} during the entire fast scan. No source with a peak rate exceeding three standard deviations of the background rate (40 counts s^{-1}) was observed in the fast scan[2]. This corresponds to a threshold source intensity of ~0·12 counts cm^{-2} s^{-1} for a source at the centre of the field of view.

The effective area of each bank of detectors while transiting Sgr γ-1 depends on the declination of the source. It is 20, 38 and 45 per cent of the area (350 cm^2) for declinations $-37°$, $-35°$ and $-33°$ respectively. Taking into consideration the error in declination ($-35° \pm 2°$) given for the source, we take 20 per cent of 350 cm^2 as the minimum effective area. Combining the data of both banks of detectors we can set an upper limit to the strength of Sgr γ-1 in the 1·5–6 keV region as 0·4 counts cm^{-2} s^{-1}. The average efficiency of the proportional counters is 0·5 in the energy range and is insensitive to the energy spectrum of the source. Therefore

$$I_{\mathrm{Sgr}\,\gamma-1}(1{\cdot}5\text{–}6\ \mathrm{keV}) < 0{\cdot}80\ \text{photons cm}^{-2}\ \mathrm{s}^{-1}$$

This upper limit, which corresponds to three standard deviations of the background rate, is ~20 per cent of the intensity of Crab Nebula[3]. With an instrument similar to that which detected Sgr γ-1, Frye *et al.* have set an upper limit (95 per cent confidence) for γ-rays from the Crab Nebula[4].

Table 1. X-RAY AND γ-RAY INTENSITIES OF Sgr γ-1 AND CRAB NEBULA

Source	I (1·5–6 keV) (photons cm^{-2} s^{-1})	I (> 50 MeV) (photons cm^{-2} s^{-1})	α
Sgr γ-1	< 0·8	$(3 \pm 1) \times 10^{-5}$	< 2·1
Crab Nebula	4·5	$< 1{\cdot}7 \times 10^{-5}$	> 2·25

The X-ray and γ-ray flux data from Sgr γ-1 and the Crab Nebula are summarized in Table 1. The last column gives the limits for the spectral index, α, assuming that both sources have a power law photon number spectrum $E^{-\alpha}\, dE$ from X-ray to γ-ray energies. It is also assumed that Sgr γ-1 has not changed appreciably in intensity between July 1967 and February 1969. For the Crab Nebula the energy spectrum can be expressed as $7\ E^{-2}$ in the energy range 1–500 keV (ref. 3), followed by a steepening at higher energies as indicated in Table 1.

In summary, assuming that Sgr γ-1 is a discrete γ-ray source[1] we can state that it has an intensity of less than one-fifth of the Crab Nebula in soft X-rays of 1·5–6 keV. Below 50 MeV, it has a spectrum flatter than that of the Crab Nebula.

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Anorthosites in the Early Crust of the Earth and on the Moon

THE recent report from Houston[1] proposed that the lunar highlands may be composed "predominantly" of anorthosite and gabbroic anorthosite. O'Hara has therefore suggested that some of the Pre-Cambrian anorthosites may be reworked slices of the Earth's primordial crust. The aim of this report is to present evidence for such a relationship between lunar and early terrestrial anorthosites. Apart from the suggestion by Olsen[2], this has not been considered in the literature on either the lunar samples or on the evolution of the Earth–Moon system. In their comparisons of the lunar anorthosites with terrestrial rocks, Wood *et al.*[3] have followed the commonly accepted idea that there are only two varieties of anorthosites—the massive Adirondack and the stratiform Bushveld types. There is also a third type, however (first recognized as such by Harpum[4] and re-emphasized by Bridgwater[5]); a layered calcic anorthosite found in some high-grade basement terrains. Anorthosites in this third group are mostly the oldest plutonic rocks in the crust with any decipherable mode of origin, having been formed more than 3,000 m.y. ago and possibly as old as 3,500 m.y., and they are extremely similar chemically to the lunar anorthosites. Indeed, the similarities are enough to suggest that these are the first terrestrial rocks to have any marked affinities with those on the Moon.

The areas in the world where these Group III anorthosites occur are (Fig. 1): (1) West Greenland[6–9], (2) Southeast Greenland[10], (3) North Lewis, Scotland[11], (4) Messina/Soutpansberg, Rhodesia[12,13], (5) Sakeny, Madagascar[14], (6) Sittampundi, Madras, India[15], and (7) Kondapalli, India[16]. The rocks belong to pre-orogenic bodies situated

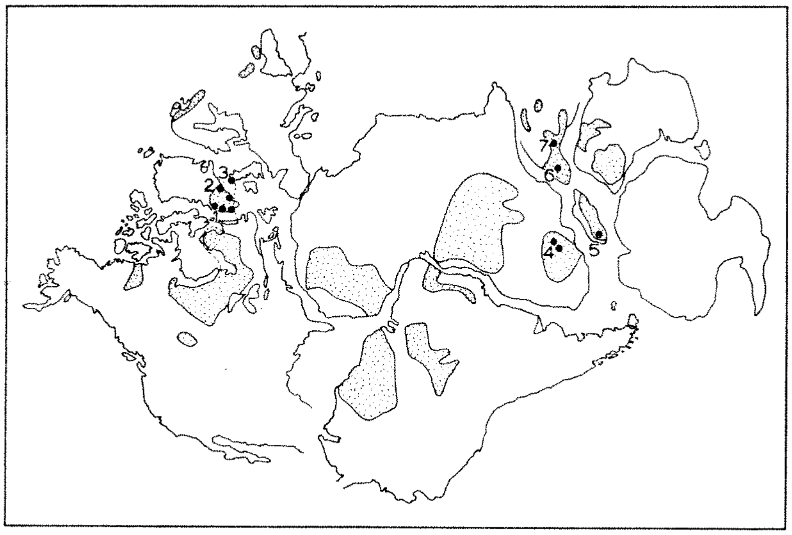

Fig. 1. Localities of early Pre-Cambrian Group III anorthosites situated within the Archaean (> 2,000 m.y.) cratons (see text). Modified after Hurley and Rand[24].

mostly in the oldest parts of the Archaean cratons, but only one radiometric age determination has been made on the actual rocks concerned: the 3,210 m.y. K/Ar date on hornblendes from an amphibolite within the Fiskenaesset complex, West Greenland[17]. A whole rock Rb/Sr or Pb isotope date on this complex might be expected to give a greater age, and therefore the time of formation of the anorthositic complex might be as much as 3,500 m.y. ago. In comparison, there seems to be general agreement[3,18] that the anorthosites on the lunar highlands formed before the mare lavas which crystallized in the period 3,600–3,800 m.y. (ref. 1).

These early Archaean Group III anorthosites have suffered the orogenic effects of one or more periods of regional metamorphism and deformation, with the result that they now occur as recrystallized tectonic pods and slices. The rocks themselves are remarkably well preserved, however, probably because an original plagioclase–orthopyroxene–clinopyroxene igneous assemblage was recrystallized isochemically into a similar metamorphic assemblage.

They are fine-grained, white granular rocks which include true anorthosites (> 90 per cent plagioclase), gabbroic anorthosites (77–90 per cent plagioclase) and anorthositic gabbros (< 77 per cent plagioclase). Where best preserved (Fiskenaesset, Sittampundi, Messina) they form extensive layered stratiform sheets with major units of anorthosite, amphibolite, peridotite and pyroxenite occurring as conformable layers (maximum thickness 6 km) in granitic gneisses. Where further deformed they survive as relic tectonic lenses, often in reworked gneisses (Lewis). There is a considerable amount of this type of anorthosite in early Archaean catazonal regions. In West Greenland, where they are best preserved, there are at least 400 km of exposed anorthositic layers[6].

The lunar and terrestrial Group III anorthosites are characterized by their calcic plagioclases, low Ti and high Ca and Al contents (Table 1).

But the chief diagnostic features of the lunar and Group III anorthosites (or associated rocks) are that they are commonly chromite bearing and that mafic minerals in both commonly have high Cr contents. All the chromites have very similar Cr : Fe ratios of about 1·0 (refs. 15, 19 and 20) which distinguishes them from Bushveld type anorthositic chromites where the ratio is 1·5. Interstitial pigeonite and augite in the lunar gabbroic anorthosite have Cr contents of 0·6 and 6·0 per cent respectively[19], while mafic minerals in some of the West Greenland rocks are rich in Cr even when there is no chromite present. The ultrabasic rocks within the Sakeny and Kondapalli anorthosites contain chromite and the Sittampundi, Fiskenaesset and Messina anorthosites are chromite layered.

Table 1. COMPOSITION OF ANORTHOSITES

	Lunar (2 samples[3])	Terrestrial (9 samples[6,13 15])
Plagioclase	An_{96-98}	mostly An_{85-100}
TiO_2	0– 0·3%	0– 0·4%
CaO	14·1–17·5%	14·0–21·0%
Al_2O_3	27·3–33·8%	25·5–33·2%

The evidence suggests that the Group III anorthosites are stratiform igneous cumulates separated from their source rocks; the source region could well be the layers of gabbroic and peridotitic differentiates at the base of the continental crust[21]. At present it is impossible to be certain how these rocks were emplaced or developed their present form. A flotation mechanism could be envisaged (see Wood *et al.*[3] and Anderson *et al.*[18] for the lunar anorthosites) involving separation of early calcic low density plagioclases from their underlying more basic differentiates, but this may meet serious problems when the associated heavy chromites are considered. The extent to which some layers were emplaced as tectonic slices into higher levels of the sialic crust is also uncertain.

There is, however, evidence that some anorthosite made its way into high structural levels to be introduced along cover-basement contacts (the Soutpansberg anorthosites formed below arenaceous sediments[13]) and even into the supracrustal deposits themselves (anorthosites occur in some greenstone belts[22]). This may mean that the Group III anorthosites remained mobile longer than their lunar counterparts and were therefore emplaced into different crustal levels at different times in the early Archaean.

Although there are many unknowns concerning these early Pre-Cambrian calcic anorthosites, it is important to recognize their existence as an integral group. On account of their extreme age, their compositional similarities with the lunar anorthosites and dissimilarities with other known terrestrial igneous rocks (chemically they are only comparable with the chromiferous grospydite inclusions in kimberlite pipes[23]), it is suggested that they are fragments of (primordial ?) layered differentiates formed early in the evolution of the Earth. This suggests that both planetary bodies went through parallel stages of development as far as the anorthosite event is concerned, and thus that the Moon may not be so dissimilar to the Earth as previously suspected.

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Anomalous Propagation of Elastic Energy within the Moon

THE records of the seismographs left on the lunar surface by the Apollo 11 and Apollo 12 missions have been discussed by Latham *et al.*[1]. From the behaviour immediately following impact of the Apollo 12 ascent stage and similar events present on the records before and after, they have inferred that energy propagation as elastic waves over large distances can be represented as a diffusive process in a low loss elastic continuum. They calculate a diffusion coefficient of ~ 1 km^2 s^{-1} and a specific dissipation function $Q \sim 3{,}600$ at a frequency of 1 Hz. If we use the time delay between the Apollo 12 impact and the commencement of the signal as the propagation time of an unscattered wave, we deduce a wave velocity of ~ 3 km s^{-1} and a scattering length $\sim 0{\cdot}5$ km. They emphasize that such a medium is quite unknown on Earth.

We wish to point out a few observations that encourage one to pursue this model for the Moon:

(i) The experiments of one of us (B. I. P.) on the measurement of Q in porous terrestrial rocks consistently show that Q increases when the ambient air pressure is reduced. At $\sim 10^{-2}$ torr, the limit of evacuation in these experiments, Q is typically five times greater than at atmospheric pressure. The effect is reversible and subsidiary experiments suggest that it is connected with an exchange of water $< 0{\cdot}05$ per cent by weight. Although indirect arguments[2] have been given that the lunar rocks did not acquire this amount of water under laboratory conditions, we suspect that the laboratory Q values of lunar rocks obtained by Kanamori *et al.*[3] (~ 10) at one atmosphere may not be representative. Obviously it will be necessary to make Q measurements on lunar rocks that have been kept under high vacuum.

(ii) The experiments of Grossman *et al.*[4] demonstrate that the surfaces of lunar material cleft under ultra high vacuum adhere when placed in contact. If the *in situ* scattering length just referred to is taken as the typical linear dimensions of separate blocks, the normal stresses present at the contact points would be more than adequate to maintain adhesion at the stress levels of the seismic wave.

(iii) Orowan[5] has suggested that stress waves through a medium may have rapidly increasing Q as the stress amplitude tends to zero. He suspects that if relative displacements of opposing crack faces approach interatomic distances the effective friction decreases to zero. This idea is perhaps not distinct from the concept of adhesion mentioned under (ii) (the maximum amplitude of the Apollo 12 signal was ~ 30 Å).

Perhaps the most important factor to emphasize is that although Q appears as a property of the medium in a continuum theory, from a structural point of view, if the material is an aggregate, the overall Q is sensitive to the state of the surfaces that make contact. Merely to postulate that the near surface region of the Moon is such an effective scattering medium probably ensures that no gas pressure can build up within it to destroy the adhesiveness of the bearing surfaces. The concept of the lossless "welded" contact mentioned by Latham *et al.*[1] seems to have some empirical support in this particular environment.

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Quasars behind Clusters of Galaxies

A SYSTEMATIC search for quasi-stellar objects associated with clusters of galaxies was started by Sandage and Miller[1], but was discontinued when no cluster was found near 3C 48. The search has been resumed recently by Bahcall, Schmidt and Gunn[2]. Bahcall[3] found twenty-one QSOs with redshifts larger than $z = 0{\cdot}2$ in the direction of Zwicky clusters. In all these cases the redshifts of the QSOs are considerably larger than the redshifts of the associated clusters. Assuming that the redshifts are cosmological, all the twenty-one QSOs would be at considerable distances behind the clusters. This suggests a simple explanation of the association of clusters with QSOs; namely, that QSOs are gravitational lens images[4].

The bending of light in the gravitational field of astronomical objects is a well established phenomenon. The recognition of the possible existence of gravitational lenses can be traced as far back as 1919, when Lodge[5] noted that a gravitational lens does not have a real focal length and produces two images. All the papers published before 1965 discuss the effect in Euclidean space, with the deflecting mass confined within a radius which is small compared with the distance by which the light rays bypass the deflector. The solution in non-Euclidean space for the situation when the light rays pass through the mass distribution of the deflector and for relativistic cosmological models was worked out by J. M. B.[4] and J. M. B. and M. F. B.[6]. Recently, Saslaw, Faulkner and Strittmatter[7] as well as ourselves[8] applied the gravitational lens effect to explain pulsating radio sources.

Every galaxy is a potential gravitational lens for all objects properly lined up behind it. The foreground galaxy acting as the lens will produce a greatly distorted image, but in amplified brightness. The image has the shape of two thin crescents, which may coalesce to a thin ring. A small galaxy of 10^{10} $M_{\odot}$ amplifies by more than five magnitudes the brightness of any object which happens to lie within 2×10^{-8} rad of the line of sight through its gravitational centre. From Wagoner's[9] calculations we know that to a redshift of $z = 2$ we see 3 to 15 per cent of all galaxies through a foreground galaxy. While not all of these intervening galaxies will be properly aligned with a suitable object to produce a large brightness amplification, the presence of a large number of gravitational lens images over the sky is self-evident. We have shown that if the object of the lens is a Seyfert galaxy, the image will be similar in all eight characteristics to the objects accepted as representative of QSOs.

It is generally assumed that at least 50 per cent of galaxies are members of clusters of galaxies. Consequently, if QSOs are gravitational lens images with 50 per cent of the lenses being cluster galaxies, we should expect eighty out of the 160 or so known QSOs to be seen behind a cluster. Unfortunately, with the usual observational techniques, clusters of galaxies can be identified as such only up to $z = 0{\cdot}3$ or, with the improved technique of Sandage and Miller, up to about $z = 0{\cdot}4$. This may explain why Bahcall has found only twenty-one QSOs behind clusters.

3C 273 is an interesting case of the connexion of a QSO with a cluster. The brightness variations of this object, monitored over eighty years, show a pattern which is similar to a random process in which a finite band of the lowest frequencies contains relatively more power than the adjacent spectrum. The dominant frequency was found to be 0·06 yr^{-1} (ref. 10). Such a brightness variation pattern is expected if 3C 273 is a gravitational lens image and the optical axis of the lens scans the object (the nucleus of a Seyfert galaxy) which itself consists of a large number of discrete secondary light sources. As a simple computation as well as a trial scan over a random source field shows, the pattern of the variations of 3C 273 can be faithfully reproduced at a scanning speed of 25,000 km s^{-1} in the frame of the object. Considering the rotation of the galaxy, that of the supergalactic system and the galactic and supergalactic coordinates of 3C 273, such an extremely high scanning speed would follow if the lens were nearer than about 12 Mpc. As 3C 273 is in the direction of the Virgo cluster, which is at a distance of 11·4 Mpc, this suggests that the lens is a compact galaxy (of the type Zw 0930-5527, or Zw 1117-5141) in the Virgo cluster. The nuclei of the two compact galaxies mentioned are small enough that from a distance of 12 Mpc their apparent diameter would be less than one arc s. The faint wisp-like nebulosity seen near the radio component A of 3C 273 could then be the irregular nebulosity often accompanying compact galaxies.

We can estimate the chance that one of the galaxies belonging to a cluster of galaxies would act as a lens for a remote nucleus of a Seyfert galaxy. The average absolute magnitude of Seyfert galaxies is $-19{\cdot}7 \pm 0{\cdot}2$ (ref. 11), so a brightness amplification of five magnitudes would be enough to raise the luminosity of the image to the range of QSO luminosities. The average distance of the tweny-one clusters in Bahcall's list is $\bar{d}_{\mathrm{d}} = 210$ Mpc, while the associated QSOs are distributed over a distance range of 780 Mpc to 6,570 Mpc (using $H = 100$ km s^{-1} Mpc^{-1}). We may write the amplification factor of a gravitational lens in the simplified form[12]:

$$A \geq 2(C/d_{\mathrm{d}})^{1/2}/\beta \text{ with } C = GM/c^2$$

Here M is the mass of the lens and β the apparent angular distance of the object from the optical axis of the lens. (The gravitational radius of the lens galaxy (10^{-2}–10^{-4} pc) is always very small compared with the distance (0·1–10 kpc) by which the light rays arriving from the object and focused on the observer bypass the gravitational centre of the lens galaxy. Consequently, all calculations on the size and the brightness of the image can use simple geometrical optics. Whenever the cosmological distance of the lens and of the object is large, the equations have to be written in the metric of the cosmological model chosen to represent the universe. In the case discussed here, when we want to compute the statistical probability of finding an image behind a cluster of galaxies, the latter being close to us, the use of Euclidean geometry suffices.) Assuming that cluster galaxies are on the average $2 \times 10^{10}\ M_{\odot}$ ($C = 10^{-3}$ pc), each of them will produce from every object located in a truncated cone of half-angle $4{\cdot}36 \times 10^{-8}$ rad behind the galaxy an image of the object brightened by more than five magnitudes. This truncated cone extends from, say, 600 Mpc to 10,000 Mpc and the number of galaxies per Mpc^3 down to an absolute magnitude of -14 is 0·47 (ref. 13), while Seyfert galaxies are estimated to be 2 per cent of all galaxies. The volume of the truncated cone behind each cluster galaxy is 2×10^{-3} Mpc^3 and thus contains $1{\cdot}9 \times 10^{-5}$ Seyfert galaxies. The chance of finding an image with the properties of a QSO behind an average cluster containing about 130 galaxies is therefore 1 in 400.

We must realize, of course, that in observable clusters a large or medium galaxy cannot act as a lens of a "quasi-stellar" object, because it would be seen in front of the image. The entire morphological configuration would not be of stellar appearance, but rather similar to that of an N-type galaxy[14]. The three types of galaxies which, at short distance from us, could act as lenses without being seen to extend beyond the star-like image are compact galaxies, dwarf galaxies and dead galaxies.

We have given already an example (3C 273) of a compact galaxy acting as a lens, where the lens is invisible in spite of being at a distance of only 12 Mpc. Zwicky's (unpublished) catalogues of compact galaxies contain two thousand objects. Considering the lower average luminosity of compact galaxies, we may infer that the number of compact galaxies is at least equal to or larger than the number of normal galaxies.

Dwarf galaxies, such as are found in the local group of galaxies (NGC 147, NGC 185, NGC 205, NGC 221, NGC 1613, NGC 6822 and those in the Sculptor, Fornax, Wolf–Lundmark and Leo systems) with luminosities below -16, would be too faint to be seen at a distance of 200 Mpc, even if their diameter is more than 1 kpc. According to de Vaucouleurs's estimate[11] the number of dwarf galaxies ($-16 < M < -11$) could be a hundred times larger than the number of normal galaxies. Assuming a ten times larger number of dwarf galaxies but at the same time a ten times smaller mass ($2 \times 10^9\ M_{\odot}$), the chance of finding a QSO behind a given cluster of galaxies is, as before, 1 : 400.

One famous puzzle of astronomy is that the total mass of a cluster of galaxies is twenty to fifty times larger than the total mass of all visible galaxies in the cluster. The same puzzle appears in cosmology where the mean density of matter in space, computed from the mass of visible galaxies (7×10^{-31} g cm^{-3}), turns out to be lower by two orders of magnitude than would follow from relativistic models of the universe. Peebles[15] has suggested that there may be many dead, invisible galaxies in the intergalactic space. Should the mass of these invisible galaxies represent the explanation of the mass discrepancy, then our estimate (1 : 400) of the chance of finding a QSO behind a cluster of galaxies would increase by a factor of between twenty and fifty.

Our final conclusion is that there is a good chance that if a QSO is behind a cluster of galaxies, this QSO is a gravitational lens image produced by a cluster member (small, compact, dwarf or dead galaxy), acting as the lens on an object (Seyfert galaxy), situated at the cosmological distance corresponding to the redshift of the QSO.

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Shape of the Crab Pulsar and its Period Fluctuations

RICHARDS *et al.*[1] have recently recorded sinusoidal variations in the arrival times of radio pulses from NP 0532 with a period of about three months and an amplitude $\Delta\tau = 6 \times 10^{-4}$ s. This effect can be interpreted in terms of genuine sinusoidal variations of the circular frequency of the Crab pulsar

$$\Omega(t) = \Omega(1 + A \cos \omega t) \qquad (1)$$

where $\Omega \simeq 200$ s^{-1}, $\omega \simeq 10^{-6}$ s^{-1}, $A \simeq 10^{-10}$. Equation (1) can be explained by the widely accepted oblique rotator model[2-4] simply by investigating an interesting consequence of the possible ellipticity of the neutron star. To account for the history and energy balance of the Crab Nebula[3], an upper limit must be placed[4,5] to this ellipticity, such that the gravitational quadrupole radiation dominates the magnetic dipole radiation only in the early life of the pulsar. It turns out that the explanation given here for the anomalies in the pulse arrival times provides in the end an even stronger limit to the deviations from spherical symmetry allowed in a neutron star.

In the oblique rotator, because of the emission of low frequency electromagnetic waves, a non-conservative torque is applied to the neutron star. The torque is responsible for slowing down[4] the rotation at a rate in good agreement with the observations, and probably for aligning the magnetic axis with the rotation axis (F. C. Michel at the Princeton pulsar meeting, November 1969). Both effects are secular (with time scales of thousands of years for the Crab pulsar), so that over periods of the order of months, the angular momentum vector **M** of the pulsar may be considered constant in direction as well as in magnitude. The pulsar accordingly behaves as a free top.

The model assumed here for NP 0532 is a slightly aspherical ellipsoid instead of a perfect sphere. Examples of such configurations in rotating bodies are well known in Newtonian gravitation (Jacobi ellipsoids) and no *a priori* argument (except for a lifetime which is possibly much too short because of gravitational radiation) can rule out their existence in general relativity. The motivation adopted here, however, for the lack of axial symmetry is magnetic, not rotational. Let x_1, x_2 and x_3 be the three principal axes of inertia of the neutron stars and let the corresponding moments of inertia satisfy the inequalities

$$I_1 < I_2 < I_3 \qquad (2)$$

With the magnetic field axis along the direction of greater elongation, x_1, let **M** be at a small angle with x_3. In other words, let the rotation be nearly around x_3, but not exactly (off axis rotation has already been investigated in connexion with other phenomena in pulsars[6]). An oblique rotator is thereby realized, the free motion of which is well known[7]. A new frequency, ω', is associated with the system in addition to the main frequency of rotation, $\Omega \simeq \Omega_3$

$$\omega' = \Omega \varepsilon_1 \varepsilon_2 \qquad (3)$$

where

$$\varepsilon_1^2 = \frac{I_3}{I_1} - 1,\ \varepsilon_2^2 = \frac{I_3}{I_2} - 1 \qquad (4)$$

are two small quantities. ω' appears in a variety of effects: in particular, it turns out[7] that the components of **M** along x_1 and x_2 are given by

$$\begin{aligned} M_1 &= aM\varepsilon_2 \cos \omega' t \\ M_2 &= aM\varepsilon_1 \sin \omega' t \end{aligned} \qquad (5)$$

where a is the small-amplitude constant related to the mean square angle θ between **M** and x_3 by

$$a^2 = \frac{2\theta_2}{\varepsilon_1^2 + \varepsilon_2^2} \qquad (6)$$

Obviously Ω_3 differs from $|\mathbf{\Omega}| = \Omega$ for terms of the order a^2, which can be evaluated by means of the appropriate Euler's equation. By using equation (5), one finds that

$$\Omega_3 = \Omega \left[1 - \frac{1}{4} a^2(\varepsilon_1^2 + \varepsilon_2^2) + \frac{1}{4} a^2(\varepsilon_1^2 - \varepsilon_2^2) \cos 2\omega' t \right] \qquad (7)$$

The constant of integration of order a^2 is really immaterial: the choice in equation (7) satisfies $M_1^2 + M_2^2 + M_3^2 = M^2$. The kinetic energy of rotation is thus

$$K = \frac{1}{2} I_3 \Omega^2 (1 + a^2 \varepsilon_1^2 \varepsilon_2^2)$$

According to equation (7) the ellipsoidal pulsar does not rotate on itself at a fixed rate $\Omega_3 < \Omega$, but slower or faster with frequency $2\omega'$, which may, of course, account for equation (1). The assumption $\varepsilon_1 \neq \varepsilon_2$, or relations (2), is then crucial, whereas a flattened spheroid with axial symmetry would not be adequate. It is assumed here that, although ε_1^2 and ε_2^2 are mainly rotational deformations, $(\varepsilon_1^2 - \varepsilon_2^2)$ is determined by magnetic effects.

For the Crab pulsar $\omega/\Omega \simeq 10^{-8}$, so that in order to justify $\omega \simeq 2\omega'$ one must have from equation (3) $\varepsilon_1 \varepsilon_2 \simeq 10^{-8}$, or

$$\varepsilon_1^2 \text{ and } \varepsilon_2^2 \simeq 10^{-8} \qquad (8)$$

The rotational deformations of a general relativistic neutron star have been evaluated under the assumption of axial symmetry[8]. For an ellipsoid such computations turn out to be forbiddingly difficult. In a Newtonian approximation every fluid model will have rotational deformations of the moment of inertia, $\Delta I/I \simeq \varepsilon_1^2 \simeq \varepsilon_2^2$ of the order[9] of $\Omega^2/(2\pi G \rho_c)$. This implies for our case

$$\varepsilon_1^2 \text{ and } \varepsilon_2^2 \simeq 10^{-4} \qquad (9)$$

rather than equation (8).

Strictly speaking, the rotational deformations in a general relativistic star are to a certain extent smaller than in their Newtonian counterpart[10] (because the dragging of the inertial frame implies, among other things, a reduction of the centrifugal force[8]). This effect can hardly be enough to reconcile equations (8) and (9), however.

If the present explanation for the period fluctuations proves correct, therefore, the discrepancy between equations (8) and (9) will by itself point to the existence of strong forces in a neutron star forbidding relatively wide deformations. A very thick solid crust[11] might perhaps be adequate. The geometrical deformations, $\Delta R/R$, implied by equation (8) are exceedingly small, the equatorial radius being only 10^{-2} cm longer than the polar radius. In a sense it is gratifying that the recent quake[12,13] on the Crab pulsar implies[11] only a minor crust readjustment of the order of 10^{-3} cm.

In terms of the assumed magnetic perturbation $\delta_M \ll 1$

$$\varepsilon_1^2 = \varepsilon_2^2 (1 + \delta_M) \qquad (10)$$

the magnitude of the variable part of the quadrupole moment is clearly $D \simeq I_3 \varepsilon_2^2 \delta_M$. The corresponding gravitational radiation energy loss[14] becomes

$$-\frac{dK}{dt} \simeq \frac{G}{c^5} (I_3 \varepsilon_2^2 \delta_M)^2 \Omega^6 \simeq 10^{28} \delta_M^2 \text{ erg s}^{-1}$$

within an irrelevant numerical factor. This is so small in comparison with the energy requirement of the Crab Nebula and assumed magnetic dipole loss[2-4] as to make the gravitational damping totally insignificant at the present time. No contradiction arises therefore with the general model (see also ref. 15).

By making use of equations (6), (7) and (10) the amplitude A in equation (1) becomes

$$A = \frac{1}{2}\,\theta^2\,\frac{\varepsilon_1{}^2-\varepsilon_2{}^2}{\varepsilon_1{}^2+\varepsilon_2{}^2} = \frac{1}{4}\;\theta^2\delta_M \qquad (11)$$

For a field of 10^{12} G the magnetic effects are likely to be rather superficial. They may be calculated within a certain model[4] or, very roughly, simply by taking δ_M equal to the ratio of the magnetic to the ordinary pressure. For an indicative density of 10^9 g cm^{-3} in white dwarf material, $\delta_M \simeq 10^{-4}$. This in turn reproduces from equation (11) an amplitude $A \simeq 10^{-10}$ for a sensible deviation $\theta \gtrsim 10^{-3}$.

Much more remains to be seen before definite conclusions can be reached, but it seems that the phenomenon in itself emphasizes the precision of the pulsar clock and supports the notion[16] of a beacon on the neutron star surface rather than farther out, accurate timing being harder in the second case. Our explanation of equation (1) is appealing because it does not require new assumptions about pulsar structure and it has a considerable flexibility.

A difficulty, however, may be that the present state of the crust theory does not seem to yield the required stiffness (equation (8)). It is also possible that the frozen crust retains much of the flattening of the original fluid configuration.

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Possible Clues to the Early History of the Solar System

ONE problem in connexion with the nucleosynthesis of the light elements, D, Li, Be and B, has been to decide whether preplanetary matter at an early stage of the solar system was intensely irradiated by solar particles. First put forward by Hayakawa[1] and Fowler, Greenstein and Hoyle[2], and later modified by others[3-5], this notion has motivated searches for anomalies in the isotopic abundances of several elements in meteorites[6-16]. Within experimental accuracies, however, these isotopic abundances were identical with those of terrestrial matter except for lithium[11,13,16], and because of recent theoretical considerations the tendency is to abandon the irradiation mechanisms of Fowler, Greenstein and Hoyle[17,18]. But it seems to us worthwhile to continue the study, on one hand by improving the experimental accuracy, and on the other by looking for abundance anomalies caused by other types of nuclear reactions.

According to Mitler, the mean proton energy of 570 MeV assumed by Fowler, Greenstein and Hoyle is too high[3], based on recent observations of solar flare particles. Hayakawa has also used, in his latest article[5], the spectra of contemporary solar particles. In this case isotopic anomalies in the product nuclides from high energy nuclear reactions such as ^{50}V or ^{54}Cr would be less probable. The neutron-induced reactions will also be less important because of the lower neutron production with softer primaries. We therefore looked for other nuclear reactions induced by low energy protons and alpha particles and found that ^{138}Ba(p,n)^{138}La and ^{180}Hf(p,n) ^{180}Ta are the most suitable, because ^{138}La and ^{180}Ta are extremely low in cosmic abundance compared with the respective target nuclides ^{138}Ba and ^{180}Hf. (Brancazio *et al.*[19] have investigated similar reactions, but their discussion refers to bombardment in stellar surfaces.) Table 1 shows the results of our calculations for these two reactions as well as other possible side reactions that may give the same product nuclei. For the calculation of average cross-section $\bar{\sigma}$, we used Barbier's semi-empirical $\sigma(E, AT)$ function[20] as no measured excitation functions are available. The shape of the integral proton spectrum was assumed to be $\varphi(>P) = \varphi_0 \exp(-P/P_0)$ according to the solar flare observation[21], where φ is the total flux for the event; P is the rigidity (MV/nucleon); and P_0 the characteristic rigidity for the solar event, here taken to be 100 MV. For the neutron spectrum in the range 2 to 20 MeV we used the differential form $\varphi = k(1 + 0{\cdot}01\ E^{-1} + 1{\cdot}1 \times 10^{-5}\ E^{-2})$ given by Arnold *et al.*[22], where E is in BeV. The abundance ratios of product nuclide to target nuclide in the preplanetary matter were taken to be the same as the cosmic abundances for non-volatile elements and Cameron's new cosmic abundances were employed[23]. (The product nuclide here means the same nuclide species present in preplanetary matter as the product of the nuclear reaction on the assumed target.) The last column of Table 1 shows a measure of the sensitivity of each reaction, that is, the integrated flux of irradiating particles that may cause a 1 per cent relative anomaly in isotopic abundance ratio. The calculation assumes uniform irradiation in the same way as done by Burnett *et al.*[9] for ^{40}K. In order to compare with other indicator elements for proton irradiation, we calculated the corresponding total flux φ_0 for each reaction, assuming the same integral rigidity function for the event. Table 2 shows the values of φ_0 with $P_0 = 50$, 100 and 200, covering the characteristic rigidity for present day solar flares. It can be seen that ^{138}La and ^{180}Ta are the most sensitive

Table 1. NUCLEAR REACTIONS POSSIBLY GIVING RISE TO THE ISOTOPIC ABUNDANCE ANOMALY OF ^{138}La AND ^{180}Ta

Reaction	Product/ target	σ (mbarn)	Integrated fluxes for 1 per cent anomaly (cm^{-2})
^{138}Ba(p,n)^{138}La	$9{\cdot}5 \times 10^{-5}$	200 (10–50 MeV p)	$4{\cdot}8 \times 10^{18}$p
^{139}La(n,2n)^{138}La	$8{\cdot}9 \times 10^{-4}$	500 (2–20 MeV n)	$1{\cdot}8 \times 10^{19}$n
^{139}La(p,pn)^{138}La	$8{\cdot}9 \times 10^{-4}$	70 (10–50 MeV p)	$1{\cdot}3 \times 10^{20}$p
^{138}Ce(n,p)^{138}La	0·11	< 100 (2–20 MeV p)	$> 1{\cdot}1 \times 10^{22}$n
^{180}Hf(p,n) ^{180}Ta	$4{\cdot}8 \times 10^{-5}$	70 (10–50 MeV p)	$6{\cdot}9 \times 10^{18}$p
^{181}Ta(n,2n)^{180}Ta	$1{\cdot}2 \times 10^{-4}$	500 (2–20 MeV n)	$2{\cdot}4 \times 10^{18}$n
^{181}Ta(p,pn)^{180}Ta	$1{\cdot}2 \times 10^{-4}$	50 (10–50 MeV p)	$2{\cdot}4 \times 10^{19}$p
^{180}W (n,p)^{180}Ta	$1{\cdot}25 \times 10^{-2}$	< 50 (2–20 MeV n)	$> 2{\cdot}5 \times 10^{21}$n

Table 2. SENSITIVITY OF INDICATOR NUCLIDES FOR PROTON IRRADIATION

Indicator nuclide	Experimental limit on abundance variation (per cent)	Integrated fluxes for effective proton energy range: Energy E (MeV)	Rigidity P (MV)	$\varphi(P)$†	φ_0(cm^{-2})*: $P_0=50$	$P_0=100$	$P_0=200$
^{7}Li/^{6}Li	2	>75	>376	1×10^{18}	2×10^{21}	4×10^{19}	7×10^{18}
^{36}S	0·1	>1000	>1680	10^{20}	4×10^{34}	3×10^{27}	4×10^{23}
^{54}Cr	1	>30	>235	3×10^{19}	3×10^{21}	3×10^{20}	1×10^{20}
^{40}K	1	>500	>1100	2×10^{19}‡	7×10^{28}	1×10^{24}	5×10^{21}
^{50}V	1	>100	>440	$2·5\times10^{17}$	2×10^{21}	2×10^{19}	2×10^{16}
^{138}La	1	50–10	305–130	5×10^{16}	6×10^{19}	2×10^{19}	$1·5\times10^{19}$
^{180}Ta	1	50–10	305–130	7×10^{18}	9×10^{19}	3×10^{19}	2×10^{19}

* φ_0 has essentially the same meaning as $\Delta\varphi$ for the solar flare spectrum in Table 3 of Balsiger, Geiss and Lipschutz[10]. The difference is that the latter is based on the power function of energy and is integrated for $E > 10$ MeV, while the former is the total flux based on the rigidity function.

† $\varphi(P)$ for the nuclides other than ^{138}La and ^{180}Ta was taken from Burnett, Lippolt and Wasserburg[9], who had used a different proton spectrum from ours. This would not make any difference, because the excitation functions for these high energy protons are nearly flat and consequently the reaction yield is independent of the shape of spectrum.

‡ The value given by Burnett, Lippolt and Wasserburg[9] was recalculated; their calculation seems not to have taken into account the decay of ^{40}K during the past $4·5\times10^9$ yr.

for soft flares with $P_0 < 100$ while ^{50}V and lithium isotopes are the most sensitive for hard flares with $P_0 > 100$. A preliminary result on ^{50}V was reported by Balsiger *et al.*[10]. Because of our lack of knowledge about the early rigidity of the solar particles, effects of both soft and hard particles should be investigated, but because little attention has been given to the softer side, it is important to study ^{138}La and ^{180}Ta.

Another merit of ^{138}La and ^{180}Ta is that the p,n reaction is efficient only when the irradiated matter is of low density like the clouds of gas or dust assumed by Hayakawa[5] (because of the short penetration range of low energy protons). This will give us information about the limits of the state of preplanetary matter at the time of irradiation.

One of us has measured[24] the isotopic abundance of ^{138}La in the Norton County achondrite but has found no anomaly within the experimental relative error of 2–3 per cent. We are preparing a similar experiment with better instrumental accuracy and on many kinds of meteorites.

Apart from the problem of the early stage of the solar system, these reactions, when combined with ^{56}Fe(p,n)^{56}Co, will be useful for the investigation of the history of solar flare activity from the lunar surface material.

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A Generalized Hoyle–Narlikar Particle Theory

Hoyle and Narlikar[1] (HN) have developed a particle (rather than a field) theory of gravitation. This is based on Mach's principle such that the mass m_a of the particle a arises from all other particles of the universe. The mass function at a general point x due to a is defined as

$$m^{(a)}(x) = -\lambda \int \tilde{G}(X,A)\mathrm{d}a$$

where λ is a coupling constant and $\tilde{G}(X,A)$ a symmetric scalar Green's function which satisfies a simple wave equation. The mass m_a of the particle a is given by

$$m_a(x) = \sum_{b\neq a} m^{(b)}(x) = -\lambda \sum_{b\neq a} \int \tilde{G}(X,B)\mathrm{d}b$$

and thus depends on position. The physical significance of the mass field is that $m^{(a)}(x)$ is the inertia contributed by the particle a to another at the point x. The inertial mass of a particle is equal to the inertia contributed to it by all the other particles. Particle equations closely related to Einstein's field equations then arise from the variation of

$$J = -\sum_a \frac{1}{2} \int m_a \mathrm{d}a$$

Islam[2] points out that his work implies that the sign in the definition of $m^{(a)}(x)$ is correctly chosen. A change of sign does not affect the geometry or geodesics of particles but makes the sign of the gravitational constant, G, which appears in the particle equations, negative. The inertial mass m, however, of an isolated particle and the inertia contributed by it are both now negative. Thus $G > (<) 0$ means $m > (<) 0$ though the possibility arises in Einstein's theory of $G > 0$ and $m < 0$ and vice versa and, in this case, the geometry is different from the usual one.

The HN theory can, however, be easily extended to allow for particles which contribute either positive or negative inertias by making the definition

$$m^{(a_\nu)}(x) = (-1)^\nu \lambda_\nu \int \tilde{G}_\nu (X,A_\nu)\mathrm{d}a_\nu,\ \nu = 1,2$$

there being no summation over the νs. λ_ν is positive for $\nu = 1,2$. This can still be termed the "mass function at a general point x due to a_ν". Both of the $\tilde{G}_\nu$ are taken to obey wave equations of the type obeyed by the $\tilde{G}$ of the usual theory, such that

$$\Box \tilde{G}_\nu + \frac{1}{6} R\tilde{G}_\nu = -\delta^4/\sqrt{(-g)}$$

$m^{(a_1)}(x)$ and $m^{(a_2)}(x)$ contribute positive and negative inertia respectively. The definition

$$m_{a_\nu}(x) = \sum_{b\nu\neq a\nu} m^{(b_\nu)}(x)$$

can be made but cannot be termed the "mass of the particle a_ν at x" because it only takes into account particles of one type of inertia and not all the particles of the universe. Instead the mass of the particle a_ν at x must now be defined as

$$M_{a_\nu}(x) = \sum_{\nu=1,2} \sum_{a_\nu} m^{(a_\nu)}(x) - m^{(a_\nu)}(x)$$

This is the generalization of the physical significance of $m_a(x)$ and means that the mass of a particle is still contributed by all the other particles.

The action corresponding to that in the HN theory is

$$J = -\sum_{\nu=1,2} \sum_{a_\nu} \tfrac{1}{2} \int m_{a_\nu}\, \mathrm{d}a_\nu$$

$$= \sum_{\nu=1,2} (-1)^{\nu+1} \lambda_\nu \sum_{a_\nu < b_\nu} \int\int \tilde{G}_\nu\, \mathrm{d}a_\nu\, \mathrm{d}b_\nu$$

such that the particle equations formed by varying J with respect to g_{ij} and putting $\delta J = 0$ are

$$\left(R_{ij} - \frac{1}{2} g_{ij} R\right)\left[\sum_{\nu=1,2} \frac{(-1)^{\nu+1}}{\lambda_\nu} \sum_{a_\nu < b_\nu} m^{(a_\nu)} m^{(b_\nu)}\right] \tag{1}$$

$$= -3 g_{ip} g_{jq} T_m{}^{pq}$$

$$+ \sum_{\nu=1,2} \frac{(-1)^{\nu+1}}{\lambda_\nu} \sum_{a_\nu < b_\nu} [m^{(a_\nu)}(g_{ij} \Box m^{(b_\nu)} - m^{(b_\nu)}{}_{;ij})$$

$$+ m^{(b_\nu)}(g_{ij} \Box m^{(a_\nu)} - m^{(a_\nu)}{}_{;ij})$$

$$+ 2(m^{(a_\nu)}{}_{;i}\, m^{(b_\nu)}{}_{;j} + m^{(b_\nu)}{}_{;i}\, m^{(a_\nu)}{}_{;j} - \frac{1}{2} g_{ij} m^{(a_\nu)}{}_{;k} m^{(b_\nu);k})]$$

where

$$T_m{}^{pq} = \sum_{\nu=1,2} \sum_{a_\nu} \int m_{a_\nu}\, \delta^4 \sqrt{(-g)}\, \frac{\mathrm{d}a_\nu{}^p}{\mathrm{d}a_\nu} \frac{\mathrm{d}a_\nu{}^q}{\mathrm{d}a_\nu}\, \mathrm{d}a_\nu$$

The calculations follow the same lines as those in the normal HN theory.

This theory is obviously conformally invariant under the mapping

$$\bar{g}_{ij} = \Omega^2 g_{ij}, \qquad \overline{m}^{(a_\nu)} = m^{(a_\nu)}/\Omega$$

As in the HN theory, a smooth-fluid approximation of the theory can be made by writing

$$m_\nu(x) = \sum_{a_\nu} m^{(a_\nu)}(x)$$

in which case the particle equations become the field equations

$$\frac{1}{2} \sum_{\nu=1,2} \frac{(-1)^{\nu+1}}{\lambda_\nu} m_\nu{}^2 (R_{ij} - \tfrac{1}{2} g_{ij} R) = -3 T_{mij}$$

$$+ \sum_{\nu=1,2} \frac{(-1)^{\nu+1}}{\lambda_\nu} [m_\nu(g_{ij} \Box m_\nu - m_{\nu;ij})$$

$$+ 2(m_{\nu;i} m_{\nu;j} - \tfrac{1}{4} g_{ij} m_{\nu;k} m_\nu{}^{;k})] \tag{2}$$

The gravitational "constant" in these equations is proportional to $(m_1{}^2/\lambda_1 - m_2{}^2/\lambda_2)^{-}$ and can thus be positive, negative or infinite in different regions of space–time.

HN have shown how a particular conformal frame can be chosen in the case of the smooth-fluid approximation equations in which G is constant and that in this frame the Einstein equations result. Here there are two frames in which, after the mapping $\bar{g}_{ij} = \Omega^2 g_{ij}$ is made, $\bar{G}$ is constant. The first frame is when $\overline{m}_1$ and $\overline{m}_2$ are both constants, in which case Einstein's equations result. The second frame is when this is not so and Ω is now chosen so that $\overline{m}_1{}^2/\lambda_1 - \overline{m}_2{}^2/\lambda_2$ is constant and the field equations

$$R_{ij} - \tfrac{1}{2} g_{ij} R = -(6G/\alpha)(T_{mij} + S_{ij})$$

result. Here

$$S_{ij} = (\alpha/G)(\theta_i \theta_j - \tfrac{1}{2} g_{ij} \theta_k \theta^k)$$

$$\theta = \ln A[m_1/\sqrt{\lambda_1} + m_2/\sqrt{\lambda_2}]$$

$\theta_i \equiv \theta_{;i}$, α and A are constants and the bars have been dropped. These field equations are the scalar–tensor equations used in the HN field theory[3], the Brans–Dicke[4] theory and other theories.

Thus the most important and interesting result has been obtained that both the Einstein and scalar–tensor field equations arise in special frames under a conformal mapping of the field equations (2). As these themselves are the smooth-fluid approximations of (1), the generalized HN particle equations (1) play a very important part in that most of the particle and field equations normally used in relativistic-like theories can be derived from them.

Hawking[5] discussed an action which was a generalization of HN's action in that he introduced positive and negative gravitational charges analogous to electric charges. He also required the same number of positive and negative charges. But he did not extend the discussion to include any particle or field equations.

More details of the working and further results will be published later.

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Geophysical Implications of the Interrelationship of the Geogravity and Geomagnetic Fields

RECENTLY there has been much interest in the geophysical implications of the satellite-determined gravity field, and one suggestion is that the low-degree gravity harmonics are associated with undulations at the core–mantle boundary[1-4]. Mathematical formulation[5] does indicate that any mass anomalies at the core–mantle boundary would be observable in the Earth's external potential. Indirect evidence in favour of this hypothesis would come from a strong correlation between the gravity and geomagnetic fields, because variations in the latter are attributed to sources located in the outer core. Egyed[3] pointed out a positive correlation between the satellite geoid[6] and the geomagnetic secular variations[7], but Coode and Runcorn[8] showed that this correlation was negligible when an improved satellite geoid[9] was used. Hide[4] has proposed, however, that certain magnetohydrodynamical considerations may suggest low amplitude, long wavelength irregularities in the core–mantle interface.

If the two fields are correlated only for a limited range of frequencies, their composite representations may not show any significant relationship because of the superposition of uncorrelated components. One must study the degree correlation function which is defined as the ratio of the degree cross covariance to the square root of the product of degree variances of the two force fields. Table 1 lists this function for the satellite-determined geopotential[10,11], the geomagnetic field due to internal

Table 1. COMPARISON OF THE GEOMAGNETIC AND GEOGRAVITY FIELDS

	Degree correlation function	
n	Gravity field[10,11] versus non-dipole geomagnetic field[12]	Gravity field[10,11] versus geomagnetic secular variations[13]
2	−0·31	−0·30
3	0·23	0·87
4	0·22	0·40
5	−0·12	−0·16
6	0·05	−0·32
7	0·10	
8	−0·09	

sources[12] and the geomagnetic secular variations[13]. The pattern of the degree correlation function is more or less similar (our unpublished work) for other combinations of the recent geopotential solutions and the recent models of the non-dipole part of the geomagnetic field and its secular variations.

The degree correlation function between the anomalous gravity field and the non-dipole part of the geomagnetic field is significantly positive only for $n = 3$ and $n = 4$. For the remaining frequencies ($n > 4$), the function oscillates around zero. The positive correlation between the gravity field and the geomagnetic secular variations is therefore limited to $n = 3$ and $n = 4$ with an unmistakable peak in the third harmonic.

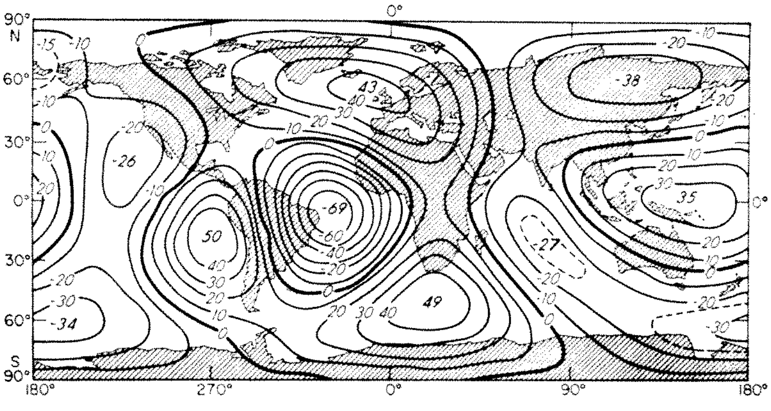

Fig. 2. Secular variations of the vertical component of the geomagnetic field arising from the third and fourth harmonics[13] for the epoch 1965.0.

Figs. 1 and 2 show the geoidal undulations and the geomagnetic secular variations respectively arising from the third and fourth harmonics. It is clear from the pattern that the two force fields are indeed correlated within the frequency range represented in the maps.

There are two explanations. (1) The long wavelength components ($n = 3$, 4) of the gravity field are associated with mass inhomogeneities in the core. (2) The sources for these components lie in the upper mantle, but their distribution is associated with geophysical parameters which affect the geomagnetic field also.

Mass anomalies in the core could be the result of either (*a*) geometrical distortion of the core–mantle boundary; or (*b*) lateral density variations in a geometrically level core–mantle interface.

Undulations of the core–mantle interface required to produce a given gravity harmonic are easily computed from formulas given by Cook[5] (Table 2, part *A*). The density differences can also be computed by a slight modification of the same formulas (Table 2, part *B*). Undulations need only be of the order of 1 to 2 km and the density difference of the order of 10^{-2} g/cm³ to produce the third and fourth harmonics in the geopotential.

This explanation, however, has important geophysical consequences which are not substantiated at present. First, if there is a differential rotation between the core and the mantle because of loose electromagnetic coupling between the two, the gravity contribution of the third and fourth harmonics will show a westward drift at about the same rate as the geomagnetic secular variations. If the north–south component of the drift is ignored, the drift will affect only the tesseral harmonic geopotential coefficients, but the time span and precision[9,14,15] of these measurements are not adequate. The only other geophysical data, pertinent to drift, are tidal records. Fig. 1 shows that, if there is a westward drift, sea level in certain areas will change by as much as 30 to 40 m over periods of 150 to 200 yr. There have been sporadic reports of long-period changes in sea level[16,17], but the tidal records do not give general evidence of changes of this periodicity and amplitude (our unpublished work).

Table 2. AMPLITUDES OF THE CORE–MANTLE BOUNDARY UNDULATIONS AND DENSITY ANOMALIES REQUIRED TO PRODUCE A GIVEN HARMONIC

A. Undulations

Degree of zonal harmonics (n)	Amplitude t_n* (km)
3	1·09
4	1·62
5	0·53

B. Density anomalies†

Degree of zonal harmonics (n)	Amplitude σ_n* (g/cm³)
for $t_n = 10$ km	
3	4×10^{-1}
4	6×10^{-1}
for $t_n = 100$ km	
3	4×10^{-2}
4	6×10^{-2}

* Let t_n denote the maximum amplitude of the undulation required to produce nth zonal harmonic coefficient C_n in the external potential of the Earth, then[5]

$$t_n = a_e \frac{2n+1}{3} \frac{\sigma}{\sigma_n} \left(\frac{a_e}{r_c}\right)^{n+2} C_n$$

where σ is the mean density of the Earth, σ_n, the anomalous density, r_c, the radius of the core, and a_e, the equatorial radius of the Earth. Note that the formula holds for $t_n \ll M_c$.

† Computed by slightly modifying the above formula for t_n.

Second, it is difficult to explain how these horizontal variations in the properties of the core–mantle boundary are maintained. The outer core is fluid and cannot support them. The only reasonable mechanism of support would be hydromagnetic (refs. 4 and 8 and our unpublished results), but the value of the toroidal magnetic field required will be about two orders of magnitude greater than the generally accepted value[8,18] at the core–mantle boundary.

We propose that the correlation is mainly due to the effect of lateral temperature variations in the upper mantle[19] on both of the force fields.

The gravity contribution of the third and fourth harmonics reaches a maximum of 15–20 mgals. To produce this gravity effect, an infinite slab of material with density contrasts of about 5×10^{-3} g/cm³ and 5×10^{-4} g/cm³ will have thicknesses of 100 km and 1,000 km, respectively. If thermal effects are responsible for producing this gravity effect, the lateral temperature anomalies in the upper mantle will be of the order of 10² °C. For most of the anomalous areas these values will be smaller.

If the surface heat flow accurately reflects the temperature distribution in the upper mantle, the maxima of heat flow should

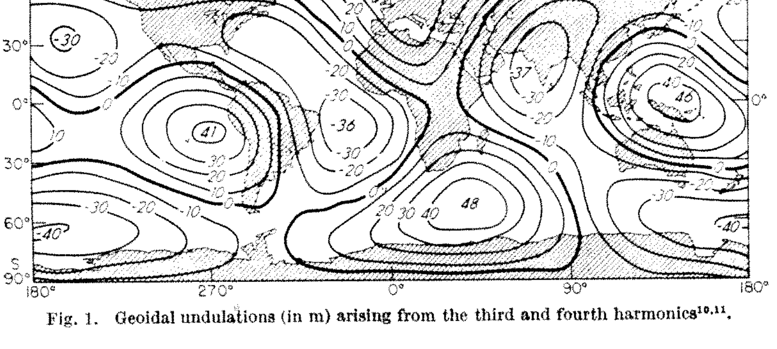

Fig. 1. Geoidal undulations (in m) arising from the third and fourth harmonics[10,11].

coincide with minima of the gravity field and vice versa. The existence of such a correlation is highly controversial[20–23]. The degree correlation function of gravity and heat flow is likely to provide a better insight into their relationship, but as yet there are not enough heat flow data. Moreover, there may be a time-lag due to low thermal conductivity of mantle material. In order to examine the thermal state of the upper mantle we have to depend on estimates of temperature distributions characteristic of different parts of the upper mantle.

Fig. 3 gives several temperature distributions[24] and shows that lateral temperature anomalies of considerable amplitude can exist in the upper mantle (assumed to be composed of olivine-rich peridotites). Fig. 4 shows that electrical conductivity of the upper mantle increases sharply with temperature[28,29]. The lateral temperature anomalies generate electrical conductivity anomalies, thus creating a spherical shell of variable electrical conductivity superimposed on a non-conducting or a uniformly conducting upper mantle (Fig. 5). The changing fields in the core induce stronger currents in the hotter and hence more conductive areas, and their associated magnetic fields oppose the propagation of the source magnetic field, thus attenuating[30] it as it passes through the shell. Hence the geomagnetic secular variations over hotter areas suffer more than normal "electromagnetic shielding". The reverse is true for colder areas. The net effect is that the gravity lows over the hotter upper mantle match geomagnetic isoporic lows and the gravity highs over the colder mantle match the isoporic highs.

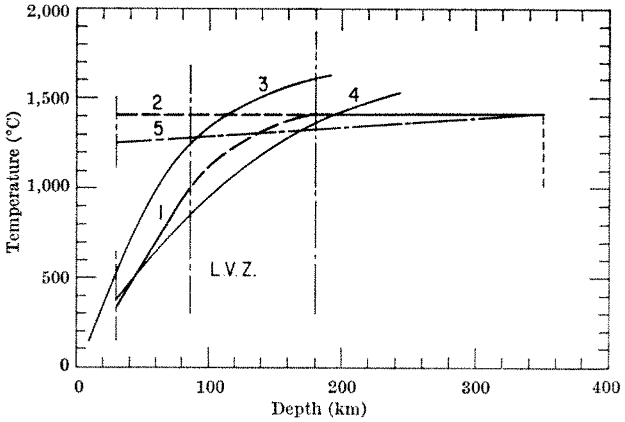

Fig. 3. Estimated characteristic temperature distributions[24] in different parts of the upper mantle. Curve 1 corresponds to temperature distribution in the thermally "normal" upper mantle based on Kanamori's[25] compressional wave velocity versus depth model for the tectonically active regions of the upper mantle and mineralogical considerations[24] governing the composition of the upper mantle. Curve 2 represents temperature distribution in the hotter upper mantle based on similar considerations. Curves 3 and 4 are representative temperature distributions in the upper mantle, under oceanic and shield areas respectively, as suggested by Ringwood[26]. Curve 5 corresponds to the adiabatic temperature distribution[27] calculated on the assumption that there is no partial melting in the low-velocity zone.

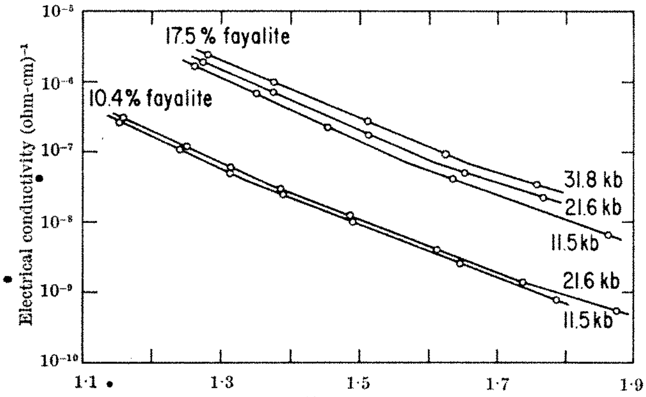

Fig. 4. Electrical conductivity of olivine as a function of temperature[29]. The mantle is assumed to be composed of olivine-rich peridotites so that the electrical conductivity of the mantle is effectively represented by that of olivine. It is also instructive to examine the theoretical dependence of the electrical conductivity σ on temperature which is given as

$$\sigma=\sigma_0\, e^{-A/T}$$

where σ_0 is a constant proportional to the number of ion-pairs per cubic centimetre, A is a constant identified with the excitation energy and the Boltzmann's constant and T is the absolute temperature.

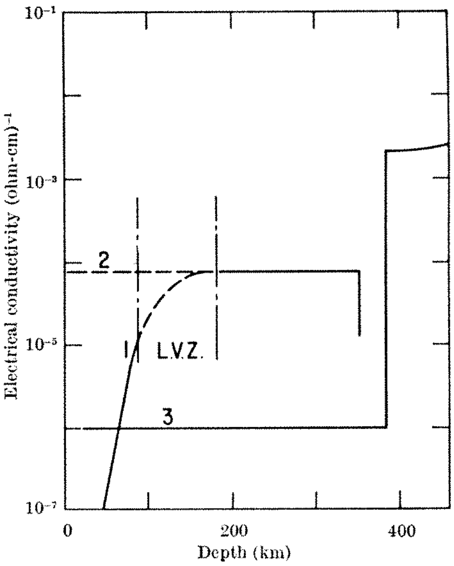

Fig. 5. Electrical conductivity distributions[24] in the upper mantle for some of the temperature distributions shown in Fig. 3. These distributions are estimated on the assumptions that: (i) the electrical conductivity data of olivine having an Fe : Mg ratio of 1 : 9, shown in Fig. 4, are representative of the olivine-rich peridotites which are generally assumed to constitute the mantle material; (ii) the pressure effects on the electrical conductivity are negligible; and (iii) the effect of partial melting on the conductivity can be ignored. Curves 1 and 2 show the electrical conductivity distribution for the temperature distributions given by curves 1 and 2 respectively in Fig. 3. Curve 3 corresponds to the electrical conductivity distribution suggested by Rikitake[18].

Recently, it was shown that the sources of the satellite-determined gravity anomalies lie in the asthenosphere[31]. It has also been shown that the positive gravity anomalies are generally associated with areas of deep-focus earthquakes, deep-sea trenches, and the young folded mountains[32,33]. Moberly and M. A. K.[34] have proposed a pattern of motions in the asthenosphere which explains the extremums of the gravity field. Considering the degree of harmonic representation used in Fig. 1 it shows good agreement with the pattern of "static", "filled-in" and "mobile" asthenosphere proposed by Moberly and M. A. K.[34]. This gives additional support to our theory that the thermal state of the upper mantle provides sources for the low-degree gravity harmonics as well as their correlation with the geomagnetic secular variations.

If the conductivity variations in the upper mantle are indeed damping the amplitudes of certain frequencies of the source magnetic field, the non-dipole field and its secular variations will be even more pronounced at the core–mantle boundary than has hitherto been suggested[35].

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Earth Normal Modes from a 6·5 Magnitude Earthquake

THE Earth normal mode spectrum, between 8 and 39 cycles per hour (c.p.h.), has been obtained with a 20 dB signal-to-noise ratio for a relatively small earthquake ($M_s = 6·5$), using a low noise broad-band vertical accelerometer of new design. The earthquake occurred in the New Hebrides (18·9° S, 169·3° E) on October 13, 1969, 06 : 56 G.M.T. at a depth of 246 km.

The instrument used[1-2] has a bandwidth extending from tidal to seismic frequencies. It uses a quartz fibre in torsion, phase sensitive capacitance detection and electrostatic feedback[3]. The internal instrumental noise was measured in the band 1–30 c.p.h. by cross-correlating[2] with another similar instrument, and was found to be the calculated Brownian noise of 2×10^{-24} g²/c.p.h. Instrumental drift is so small ($< 10^{-9}$ g/day) that it can be operated at full scale tidal gain for several months without re-zeroing.

The instrument has two outputs at present. The tidal output (Fig. 1) is flat from DC to 5·75 c.p.h., where it breaks at 20 dB/decade. The gain in the tidal channel is such that ±10 V output, or full scale, corresponds to $\pm 3·7 \times 10^{-7}$ g. The second output is a band pass filter with 40 dB gain relative to the tidal channel in a 1–30 c.p.h. band. Above 30 c.p.h. the filter output drops off at 40 dB/decade. The filter gain is such that ±10 V corresponds to $\pm 3·7 \times 10^{-9}$ g in the 1–30 c.p.h. passband. The calibration factor[4] for the instrument was obtained from a static tilt calibration using the tidal data channel and correcting for the added gain of the filter channel in the 1–30 c.p.h. passband.

The site, near Miramar Naval Air Station in San Diego, is noisy at seismic frequencies from jet engine noise, but at Earth mode frequencies (1–30 c.p.h.) the ambient power spectrum (Figs. 3 and 4) is low enough to permit the measurement of normal mode frequencies and line structure from small earthquakes.

Tidal data for a two day run are shown in Fig. 1. These are raw data which have been analogue filtered by a two-stage buffered RC filter with a 100 s time constant and digitized at 1 s intervals. The earthquake (Fig. 2) shows up as a symmetric trace superimposed on the band pass filter record, and showing the absence of nonlinear mechanical coupling within the instrument. Figs. 3 and 4 show the earthquake power spectrum from 1 to 39 c.p.h. The typical ambient power spectrum of the site was obtained from filter data corresponding to the 15 h time period AB (Fig. 1). The earthquake power spectrum was obtained from the filter data corresponding to the 15 h period CD. A small underseas earthquake occurred in the period BC, which could not be used to obtain an ambient power spectrum.

A comparison of the measured modal spectrum with the ambient power spectrum shows that there are several low order modes which can be distinguished[4] from the noise below 10 c.p.h., and that the mode structure above this frequency is real. In fact, in the region from 10 to 39 c.p.h., the signal-to-noise ratio is better than 20 dB.

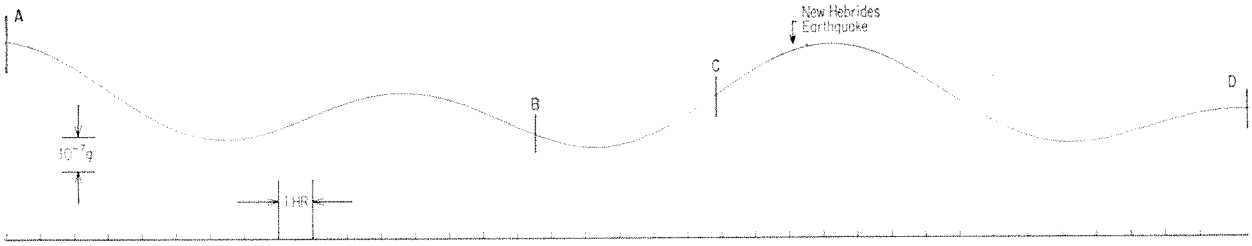

Fig. 1. Tidal channel output from 8:29:17 GMT, October 12, to 20:58:36 GMT, October 13. Filter channel output corresponding to tidal data from Section AB was used to obtain the ambient spectrum for the site. The main earthquake time series was taken from the filter channel output corresponding to section CD.

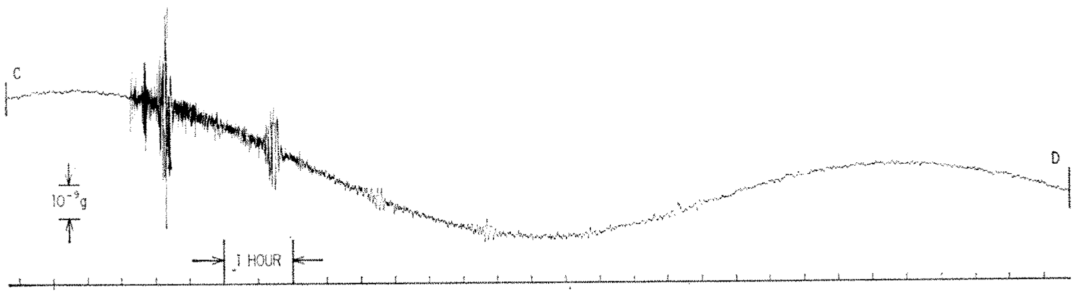

Fig. 2. Raw filter channel output corresponding to section CD of Fig. 1, showing the details of the time series for the New Hebrides earthquake.

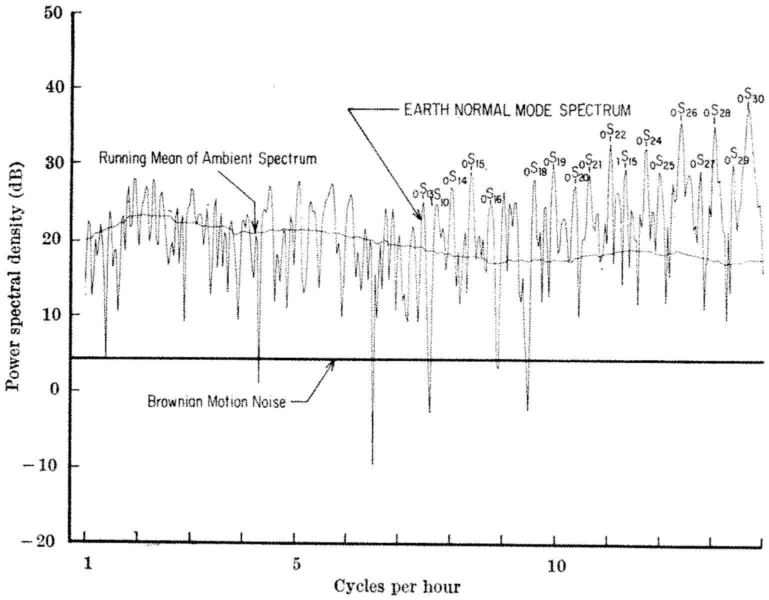

Fig. 3. Spectra from 1 to 14 c.p.h. The Earth normal mode spectrum refers to section CD in Figs. 1 and 2. The running mean of the ambient spectrum refers to section AB in Fig. 1. Brownian motion noise is shown as the heavy solid line. The 0 dB point corresponds to an absolute spectral density of $7\cdot4\times10^{-25}$ g²/c.p.h. Selected modes are identified (personal communication from Freeman Gilbert and ref. 5).

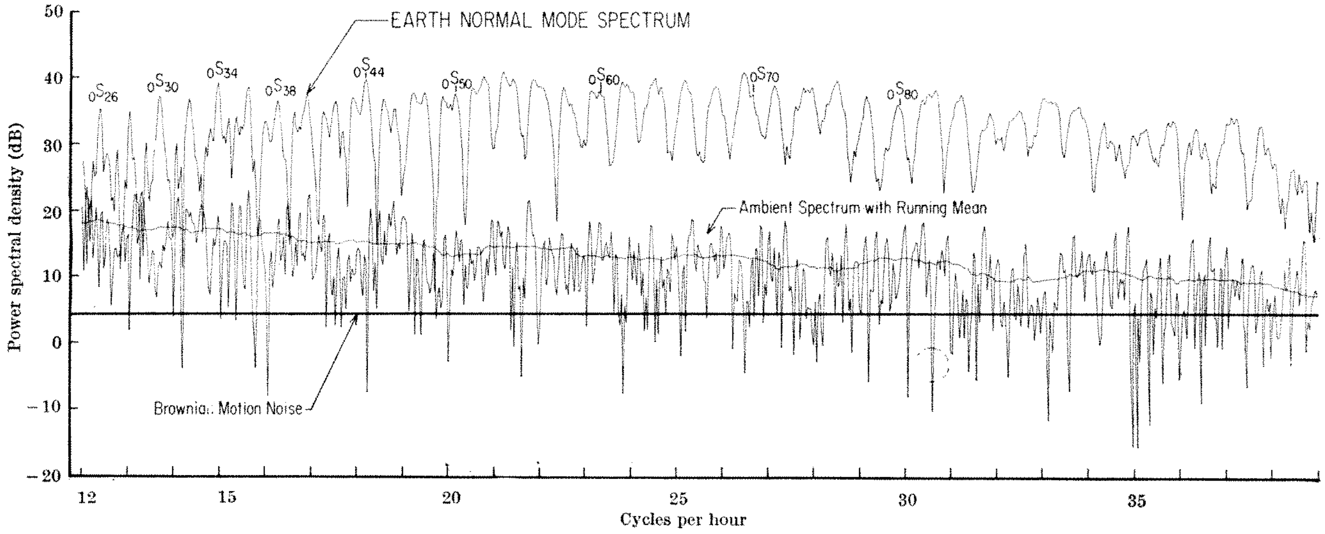

Fig. 4. Spectra from 12 to 39 c.p.h. The Earth normal mode spectrum refers to section CD in Figs. 1 and 2. The ambient spectrum and its running mean refer to section AB in Fig. 1. Brownian motion noise is shown as the heavy solid line. The 0 dB point corresponds to an absolute spectral density of $7\cdot4\times10^{-25}$ g²/c.p.h. Selected modes are identified (personal communication from Freeman Gilbert and ref. 5).

Although not shown in the figures, spectra for both the earthquake and the ambient noise were taken up to a frequency of 120 c.p.h. Up to this frequency, the earthquake spectrum shows considerable structure at least 20 dB above the ambient spectrum. At a more favourable site, the ambient noise would be lower and would enable data to be gathered over a longer time for increased mode resolution. For this reason, instruments which have a considerably lower Brownian motion limit than the one used here are under construction.

The good signal-to-noise ratio (20 dB) found here shows that normal mode and earthquake source data can now be obtained for a much larger class of earthquakes than previously possible. The smaller earthquakes can be examined for source structure and the large ones, with their consequently longer data runs, can be used to resolve modal line structure. A more complete treatment analysing several earthquakes is in preparation.

We thank James Brune for his help in identifying various features of the earthquake, Richard Haubrich for his assistance in the data handling and analysis and Freeman Gilbert for his assistance in identifying normal modes. This research was supported by the Advanced Research Projects Agency of the Department of Defense and was monitored by the Air Force Office of Scientific Research.

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Diamond Accumulation in the Upper Mantle

THE present high gradient of density in the upper mantle provides neutral buoyancy zones in which certain minerals may have been accumulated in the past or to which they may still be migrating. The necessary condition for progressive accumulation in such a zone is that the mineral concerned has opportunities of rising or sinking relative to its surroundings with a velocity which, averaged over long periods, is great enough to overcome the bulk convective drift if such occurs. Frank[1] has pointed out the high heat transfer capability of a small volume fraction of melt fluid percolating through the bulk, compared with bulk convection. With percolation active, it is likely that a fluctuating distribution of migration and local concentration of fluid has been the dominating pattern of events in the mantle, at least down to 500 km, during a large fraction of the Earth's history. Hence it is unlikely that any portion of the mantle above this level has escaped epochs during which fluid has percolated through it. If during such an epoch of involvement in local fluidity the fluid fraction becomes sufficient to lower the effective viscosity to within an order of magnitude of that of a normal homogeneous melt, then insoluble crystals suspended in the partial melt, rising or falling under buoyancy forces, could well attain velocities a million-fold greater than any plausible bulk convection velocity of the mantle in which the localized partial melt is embedded.

Table 1. DIAMONDS IN THE UPPER MANTLE

(i) Depth (km)	(ii) Pressure (kbar)	(iii) Mantle density ϱ_M (g cm^{-3})	(iv) Diamond density less mantle density $\varrho_D-\varrho_{M_3}$ (g cm^{-3})	(v) Viscosity velocity product ηv (dyne cm^{-1})
100	33	3·32	0·18	2·2
200	65	3·35	0·16	2·0
300	98	3·44	0·08	1·0
400	130	3·62	−0·09	−1·1
500	170	3·89	−0·34	−4·3
600	210	4·14	−0·57	−7·2

Consider diamond, for if migration towards a neutral buoyancy zone has acted to concentrate any mineral at all, then it would be expected to have operated on this species. Diamond is the physically stable form of carbon from a depth of about 100 km down to 1,200 km or more[2,3] and its neutral buoyancy level, as shown by Table 1, is at about 350 km depth. In Table 1, columns (ii) and (iii) give the pressure and mean mantle density at the depth stated in column (i) according to Solution II of Birch[4] which is close to Bullen's *A*(*i*) model[5]. Column (iv), which lists the difference between the density of diamond (ρ_D) and mantle (ρ_M), makes use of Berman's calculations of the density of diamond at zero pressure and elevated temperature[2] together with the value $1{\cdot}75\times10^{-4}$ kbar^{-1} as the compressibility of diamond. The variation of temperature with depth adopted is the mean of the "oceanic" and "continental shield" distributions of Tozer[6]; it suggests about 1,900 K at the neutral buoyancy depth of 350 km. The final column (v) is based on special assumptions. It takes a typical diamond to be one having a volume of 0·1 cm^3, or a mass of 0·35 g ($1\frac{3}{4}$ carats), and to be of octahedral shape. The distance from centre to corner of the octahedron is used as the effective particle radius in the Stokes formula for drift velocity v cm s^{-1} through a medium of viscosity η poise.

It will be seen that velocities of drift towards the 350 km level exceed 1 km yr^{-1} at distances greater than 50 km above or below this level when the viscosity is 300 poise or less. If a diamond of the size assumed becomes but once in every 10^6 yr incorporated for a brief period in a local accumulation of partial melt extending vertically through a depth of order 1 km, then the aggregate of possible vertical drifts would be sufficient to produce, say, a descent from 100 km to 250 km in about 10^8 yr.

Does any evidence point to the existence of a diamond reservoir at a depth of 350 km? Very long storage at about 1,900 K would probably provide good conditions for developing the distribution of nitrogen impurity platelet precipitate commonly observed in diamond, but much shorter exposures to high temperature (such as would be experienced in a rapidly rising and expanding melt) might be sufficient[7,8]. Rather, perhaps, one should enquire where does the Earth's store of carbon reside? In meteorites and in the Earth's lithosphere there is about one carbon atom to every 300 silicon atoms[9]. But the majority of meteorites seem to have been derived from small bodies in which negligible internal pressures due to gravity would have been developed, and much carbon may have escaped as volatiles. In bodies the size of the Moon and greater, interior pressures rise well into the diamond-stable region of the carbon phase diagram, making possible the retention of carbon as dense and unreactive diamond. Allowing the Earth's mantle no greater abundance of carbon than has been retained by meteorites, and assuming such carbon to be principally in the form of diamond, then if most of the diamond has migrated to within 100 km of its neutral buoyancy level, diamond concentrations in this zone might exceed one per cent.

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Fitting, Fretting and Imprisoned Boulders

THE occurrence on rocky coasts of what Shelley[1] aptly calls "fitting boulders" is, as he says, not mentioned in standard works on shore processes; but the late Professor J. A. Bartrum discussed such boulders with me in 1934 and used the term "fretting" in relation to the development of common interfaces between adjacent boulders. Baker[2] described an example near Cape Paterson in Victoria (Australia) using the term "imprisoned rocks", and indeed they are not uncommon on the Victorian coast. I have observed them at Beaumaris among blocks of soft red Tertiary sandstone derived from collapses of the cliff face, also at Sorrento in Pleistocene calcareous aeolianite, and again around Lorne and Cape Paterson where the rocks are Mesozoic arkoses. They occur also at St David's (South Wales). Shelley's descriptions afford a basis for comparison.

Among the New Zealand examples one type comprises agglomerations of boulders piled together and fitting closely, the other being large isolated boulders which fit against the bedrock base. This is also true in Victoria. In both areas fitting boulders lie about or above mean sea level, but certainly in Victoria in a zone which is at present subject to wave action if only during storms. Shore platforms, on which the boulders occur as large isolated blocks

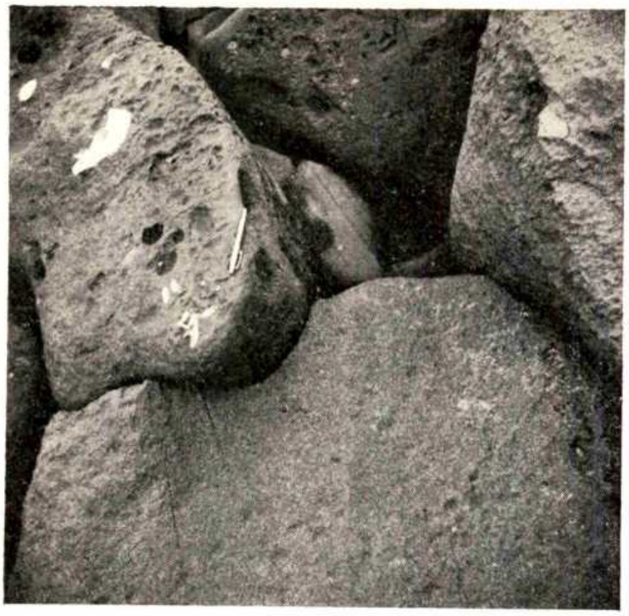

Fig. 1. Fitting boulders of Mesozoic arkose at base of cliff, Point Castries, Victoria. Blocks are derived from roadworks on hill above.

or piled against the cliff base, are normally free of boulders or cobbles, and it was the unusual sight of a shore platform dotted over with blocks of rock up to some 2 cubic yards in size that led me to investigate the matter. At Point Castries near Lorne roadworks had been carried out, and large volumes of broken rock (arkose and mudstone) had been recently dumped onto the shore platform. The finer grades or rubble had already been washed away leaving only the largest blocks scattered on the platform, and a residual pile aligned along the base of the cliff including blocks of all sizes from 3–4 feet down to a few inches across, the blocks resting as described by Shelley and by Baker intimately against each other with closely fitting interfaces (Fig. 1). At the base the shore platform had been eroded to a depth of an inch or two, forming a gutter beneath the boulder pile, which later at first sight resembled a storm beach except for the complete disarray of the blocks, their lack of sorting, and, of course, their environment. Indeed, those blocks that had not become interlocked had been swept away. It seems that the final action of waves of translation that cross shore platforms is to induce repeated small jigging and jogging movements among such boulders, causing them to fret against each other and against the base on which they rest.

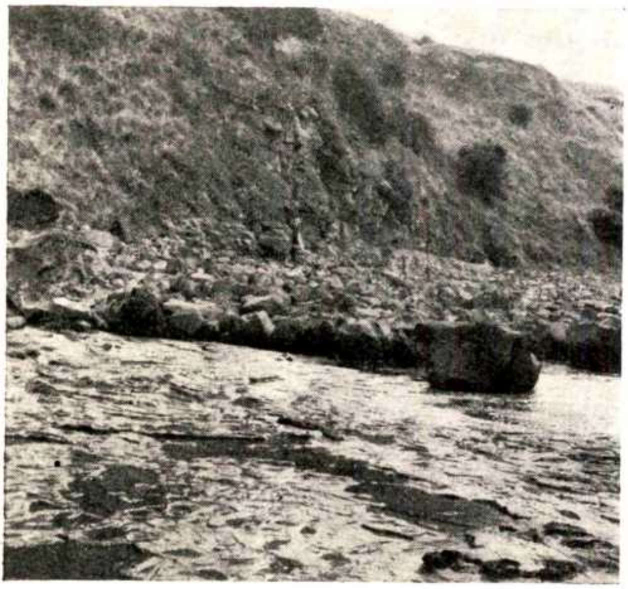

Fig. 2. Shore platform in arkose near Cape Paterson, Victoria, with a partial cover of fretting boulders derived from cliff collapse outside the photographed sector, behind the observer. Longshore drift from left to right.

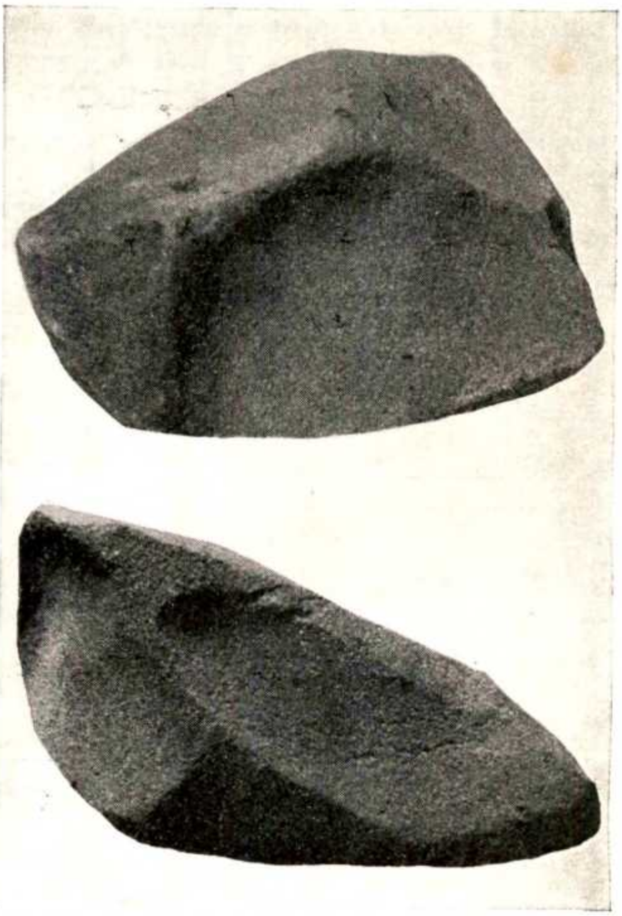

Fig. 3. Imprisoned boulders, each about 8 inches in length, removed from within a pile of fitting arkose boulders, Point Castries.

The conditions at Cape Paterson are similar, but there it is massive collapses of the cliff face that have supplied the debris. While the shore platform has been stripped bare in exposed situations, hundreds of blocks averaging about 2·5 cubic feet have been lifted and pushed along it towards the leeward end until they completely cover the platform and the wave ramp with a layer one block deep, all jostling against each other (Fig. 2). It is clear that to the limit of their capacity, waves remove such debris. Only in the gutter previously mentioned or locally beneath large residual blocks is the platform in any way affected, the removal being either rapid or completely resisted.

At all localities studied in Victoria[3] the fitting boulders originated from some special cause, which is not directly related to the processes involved in the formation of level shore platforms. In most instances the blocks derived from collapse of the cliff face. Although the undermining of cliffs results from marine processes, collapses are episodic and the sudden addition of great quantities of debris is a catastrophic event unrelated to the balance of erosion and transportation on the strand. Thus the situation with cliff falls is comparable with that of the artificial dumping of rocks at Point Castries.

Near Inverloch east of Cape Paterson, at a place called the Sheep Rocks, there is a low cliff which does not provide rock falls. The platform is, however, dotted over with large ellipsoidal blocks, some of which remain supported as mushroom rocks, others becoming detached and fretting against the bedrock base. These blocks are concretionary nodules in mudstone and arkose, released by weathering and erosion on the platform, and accordingly they are a capricious result of the marine processes that are involved in the development of a platform in the host rocks. The example at Lake Wanaka reported by Shelley, where boulders of chlorite-zone schist are derived from moraines, is comparable in that the waves on the shore are presented with ready made boulders which they themselves did not produce.

I disagree with Shelley's suggestion that crystallization of salt (or ice) is the principal agent involved in producing fitting boulders (Fig. 3). It is optically demonstrable that the boulders are moved by wave action, and the notion that long-continued very small movements are indeed responsible is inescapable. There is in Victoria, moreover, a relationship between wave force and the maximum size of fitting boulders, also with the height above mean level at which fitting boulders are to be found. This ranges from 3 to 4 feet in relatively sheltered and shallow waters of Port Phillip Bay to more than 10 feet on the exposed southern coast. One block 15 × 10 × 10 feet is fitted to the shore platform at mean sea level at Sorrento and salt crystallization is impossible for this and many other examples which never dry out. Ice formation on the shore is unknown in Victoria.

The most significant inference to be drawn from occurrences of fitting boulders is that the development of level shore platforms is not due to abrasion by waves armed with pebbles and boulders, but to other processes operating within the broad zone subject to wave attack on cliffy coasts.

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Buried Channels of the Thames Estuary

As part of a detailed and continuing study of the distribution and movements of the sediments in the area of the Thames Estuary shown in Fig. 1, we have tried to discover the thickness of the superficial deposits overlying the bedrock which is London clay for the greater part of the estuary.

More than 350 nautical miles of continuous reflexion seismic profiling from the MFV Sea Symphony were run during 1967–68 with an additional 120 nautical miles during 1969. Profile records from a further 300 miles were made available to us by kind permission of Marinex Gavel Ltd.

Equipment used in 1967–68 and for the Marinex records was a Hunting Hydrosonde 2A sparker system used at 165 J energy level. In 1969 a modified Edgerton Germeshausen and Grier sparker system was used at varying energy levels, mostly 100 and 200 J. Navigation and

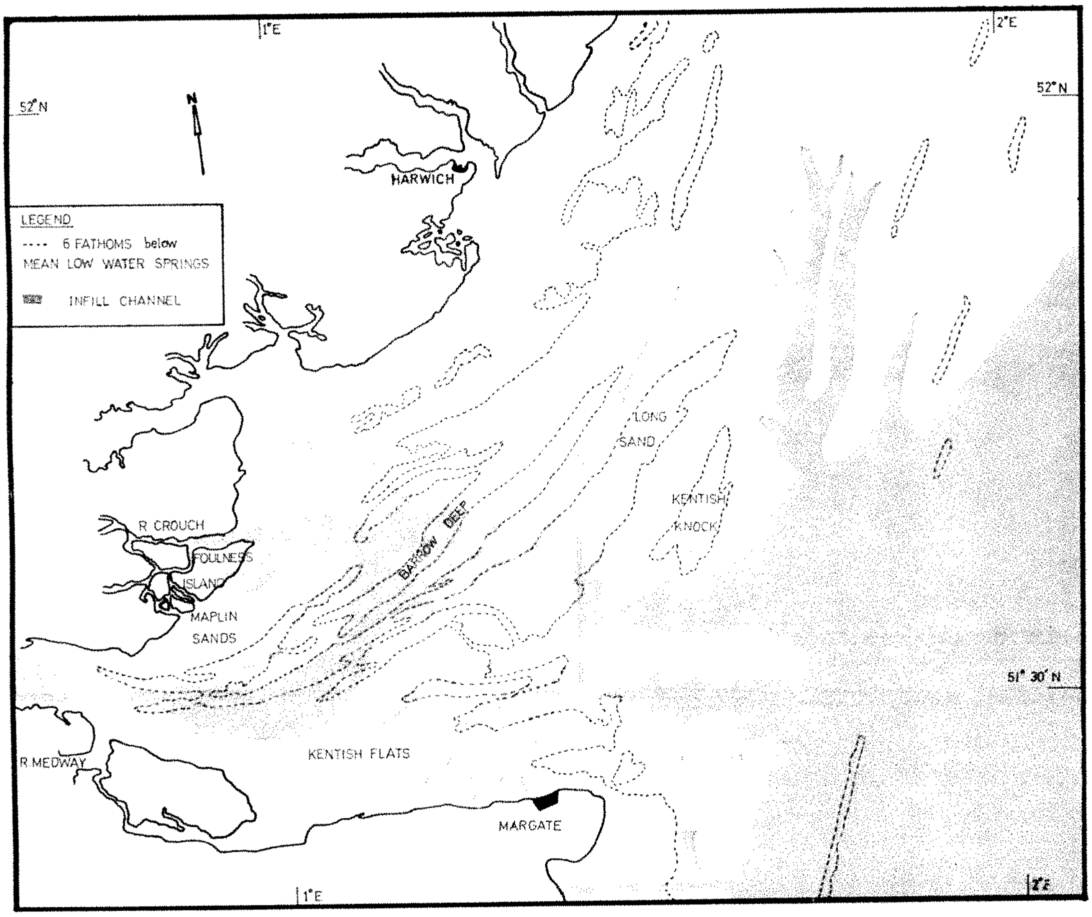

Fig. 1. The present configuration of the Thames Estuary and its main buried channels.

position fixing throughout the surveys were by Decca Navigator Mark 12. Additional information came from some deep boreholes and by using a (Shell) gravity corer in critical areas. Many hundreds of seabed samples and more than 200 miles of Kelvin Hughes MS 43 Transit Sonar records have also proved very useful.

The thickness of superficial sediment varies in the estuary from nothing or only a few inches of sand, shell or gravel to a maximum of approximately 160 feet. Much of this sediment is contained in large linear sandbanks which trend south-west–north-east, while an equal or perhaps even greater quantity infills a series of deep channels cut into the surface of the London clay and, in the extreme south around the Isle of Thanet, the chalk. The possible relationships between the present sandbank and channel topography and the buried channels have been reviewed before. It is only now that modern techniques have enabled a relationship to be established[1].

A preliminary description of these channels cut at a time when sea level was at least 200 feet lower than at present is given here; a detailed analysis of all the evidence is nearing completion and will be published later.

The main buried channel runs due east, meeting the buried channel of the River Medway in the region of the Spile Bank. This channel, now wider and deeper, continues to run east before turning almost due north under Shivering Sands. After running for about five miles in a northerly direction it meets a complex of buried river channels, the precursors of the rivers now draining south-east Essex. The largest of these, the Crouch Channel, is more than 110 feet deep under Foulness Sands and merges there with a channel which it is believed (from borehole evidence) runs across Foulness Island and then turns to run north-east across the area now occupied by the Foulness Sands.

The confluence of the main Thames buried channel and the channels of the Crouch and its tributaries occurs in the vicinity of the Barrow Sand and Barrow Deep and this channel then runs in a direction south of east under Long Sand into an area south of the Kentish Knock sandbank before turning due east. South of this, once draining the area now occupied by the Kentish Flats and the mainly submerged outcrop of the chalk of Thanet is a channel running east the tributaries of which can be largely equated with those rivers and streams at present draining this area of East Kent.

Near the Inner Gabbard and Galloper sandbanks run channels draining south which are the subject of a detailed study at the moment and seem to have a complex history.

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Precipitation of Metastable Carbonate Phases from Seawater

Because of the widespread occurrence of carbonates in marine sediments there has been a continuing interest in the mineralogy of carbonate phases that can be chemically precipitated from seawater[1-9]. The discovery of metastable carbonate minerals (aragonite and high-magnesium calcites) of non-skeletal origin occurring as cementing material in submarine sedimentary environments[10-12] has further stimulated interest in mechanisms that might explain these occurrences. Aragonites have been formed from seawater in a variety of experiments approximating natural conditions[7,8,13]. Attempts at the synthesis of high-magnesium calcites in such conditions have met with little success. We only know of three published papers in which high-magnesium calcites have been precipitated from seawater[2,5,9]. In only one of these was supporting X-ray diffraction evidence presented to document the mineralogy and in all cases the phases were produced by the addition of Na_2CO_3 or $NaHCO_3$.

The present work describes preliminary experiments involving precipitation of some metastable carbonates from seawater at room temperature using ammonium carbonate generated by a vapour diffusion technique[14], or by its *in situ* production through the enzymatic decomposition of urea with urease[15]. Because ammonium carbonate can be a natural by-product of biological activity, this suggests a mechanism by which such mineral phases might occur locally in marine conditions.

Seawater obtained from Woods Hole, Massachusetts (35 parts per thousand salinity), and at a station off the coast of Virginia (33 parts per thousand salinity) was filtered or allowed to settle. All experiments were run at room temperatures (23°–25° C) for durations of from 24 to 72 h. The precipitates were separated by filtration, washed with deionized water and dried with acetone. The dried precipitates were gently ground and examined using Debye–Scherrer X-ray diffraction powder techniques. Magnesium content of the calcitic material was estimated from the shift in the (112) reflexion[16].

To form precipitates through the vapour phase diffusion technique, 50 ml. beakers containing seawater were placed in a desiccator over solid ammonium carbonate. In seemingly identical conditions, precipitates formed from the vapour diffusion method included X-ray amorphous phases, monohydrocalcite ($CaCO_3 \cdot H_2O$), a mixture of nesquehonite ($MgCO_3 \cdot 3H_2O$) and aragonite, and magnesian calcites ranging in magnesium content from 3 to 32 mol. per cent $MgCO_3$. The high-magnesium calcites occur as very finely divided powders consisting of individual or interpenetrating wheat-shaped crystals (Fig. 1). The experimental data are summarized in Table 1.

Table 1. SUMMARY OF DATA ON CARBONATES PRODUCED FROM SEAWATER

Sample	Seawater salinity (parts per thousand)	$(NH_4)_2CO_3$ procedure	Phase precipitated	Calcite* Δd_{112}	Mol. per cent $MgCO_3$
1	33	Vapour	Transition	—	—
2	33	Vapour	Transition (recryst. aragonite + nesquehonite)	—	—
3	33	Vapour	Monohydrocalcite	—	—
4	33	Vapour	Calcite	0·076	27
5	33	Vapour	Calcite (trace of aragonite)	0·036	13
6	33	Vapour	Calcite	0·026	9
7	35	Vapour	Amorphous	—	—
8	35	Vapour	Transition	—	—
9	35	Vapour	Calcite	0·086	32
10	35	Vapour	Calcite	0·023	9
11	35	Vapour	Calcite	0·003	3
12	35	Enzyme	Monohydrocalcite	—	—
13	35	Enzyme	Monohydrocalcite	—	—
14	35	Enzyme	Aragonite + calcite	0·063	22
15	35	Enzyme	Aragonite + calcite	0·048	17
16	35	Enzyme	Aragonite + calcite	0·033	12

* Although incorporation of other cations is possible, the shift in the (112) reflexion here is considered to be entirely due to magnesium.

In the cases where nesquehonite and aragonite formed, a transition phase was commonly observed. This phase consisted of radiating clusters of sharply terminating, clear crystals. The crystals were soluble in distilled water and recrystallized in seawater or the mother liquor to nesquehonite and aragonite. Comparison of the measured *d*-spacings of this transition phase from seawater with those of the phase produced in the same manner from a 0·6 M solution of $MgCl_2 \cdot 6H_2O$ shows them to be very nearly similar (Table 2). If allowed to dry, it eventually becomes opaque

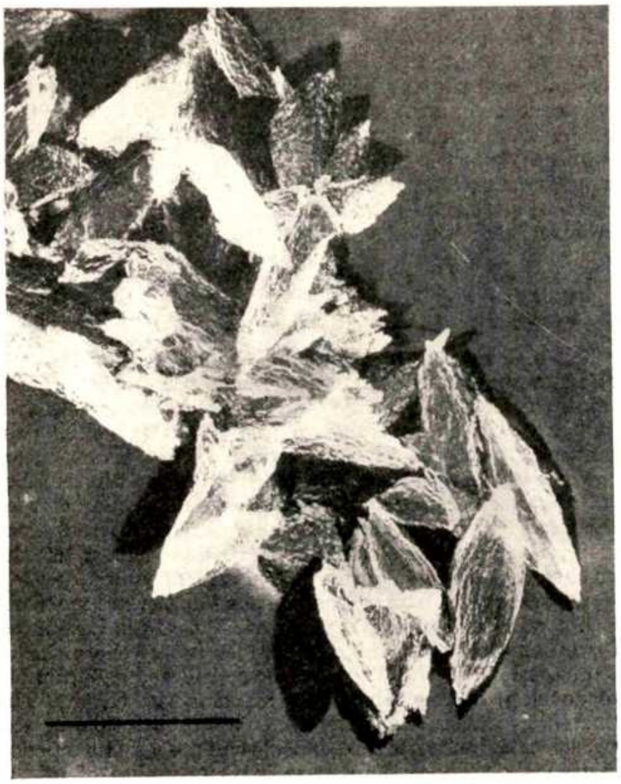

Fig. 1. Electron micrograph of magnesian calcite (9 mol. per cent $MgCO_3$) formed by vapour diffusion of $(NH_4)_2CO_3$ in seawater. Scale represents one micron.

and amorphous to X-rays. X-ray examination of calcined samples of the crystals showed strong lines for MgO and weak lines for CaO. The addition of ammonium carbonate to magnesium solutions is a common method of preparing ammonium magnesium carbonate[17], $(NH_4)_2CO_3 \cdot MgCO_3 \cdot 4H_2O$. Although we have found no published X-ray data for this compound, the physical properties together with our X-ray data suggest an impure, calcium-rich, ammonium magnesium carbonate. The presence of the larger calcium ion would account for the slightly larger *d*-spacings observed in the seawater precipitate.

In order to study precipitates involving the *in situ* enzymatic production of ammonium carbonate, 1·0 g of urea and 0·1 g of urease were allowed to react in stoppered flasks containing 100 ml. of seawater. Here the precipitates include monohydrocalcite and mixtures of aragonite and high-magnesium calcites as well as X-ray amorphous material. The magnesium content of the calcites ranged from 12 to 22 mol. per cent $MgCO_3$ and the data are included in Table 1. In no case was nesquehonite or the transition phase observed, probably because of lower concentrations of ammonium carbonate.

Table 2. COMPARISON OF X-RAY DATA OF TRANSITION PHASES PRODUCED BY PRECIPITATION OF SEAWATER AND MAGNESIUM CHLORIDE SOLUTION THROUGH VAPOUR DIFFUSION OF AMMONIUM CARBONATE

Precipitate from Woods Hole seawater		Precipitate from magnesium chloride solution	
Relative intensity	*d*-spacing (Å)	Relative intensity	*d*-spacing (Å)
Moderate	6·16	Moderate	6·15
Moderate	4·52	Moderate	4·50
Moderate	3·85	Moderate	3·83
Very weak	3·34	Very weak	3·30
Strong	3·08	Strong	3·07
Very weak	2·92	Very weak	2·91
Weak	2·79	Moderate	2·77
Very weak	2·58	Weak	2·57
Weak	2·51	Moderate	2·49
Very weak	2·40	Weak	2·39
Very weak	2·33	Weak	2·32
Weak	2·28	Moderate	2·27
Moderate	2·17	Weak	2·13
	—	Weak	2·03
Moderate	1·93	Moderate	1·92
	—	Weak	1·79
Weak	1·75	Moderate	1·74
	—	Very weak	1·71
	—	Very weak	1·65
	—	Very weak	1·58
	—	Weak	1·39
	—	Weak	1·28

Earlier work with precipitates[2,5] produced by the addition of sodium carbonate to seawater indicates that the concentration of Na_2CO_3 and the *p*H of the solution at the time of nucleation are important factors controlling the phases produced. Similarly, in the present experiments, the amount of ammonium carbonate added by diffusion or enzyme action and the *p*H of the seawater at the time the precipitate forms may determine the type of carbonate produced. Furthermore, particulate material not removed by settling or filtering may have acted as seeds and influenced the final products. Additional work should indicate the degree to which these processes may be operational in the local submarine environment where metastable carbonates of non-skeletal origin are now observed.

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Polymer Microdefects as the Centres of Destructive Cracks induced by Laser Irradiation

UNDER laser radiation separately located disk-shaped cracks are formed in irradiated transparent dielectrics such as polymethylmethacrylate (PMMA), polystyrene (PS) and polycarbonate (PC). To explain this for materials with small absorption coefficients the sample must have particular sorts of microdefects and we now consider the size, location, structure and properties of these microdefects.

The cleaving effect of the gas is the cause of these destructive cracks[1,2]. Uniform compression of the sample can obviously prevent the crack development, and the further from the focal points of the irradiation the less probable is the formation of a crack. Uniform compression will first of all prevent the formation of remote cracks, as shown by experiment[3]. From the results of these experiments it is possible to determine the minimum gas pressure (180 ± 10 atmospheres) in microdefects required to cleave the sample and lead to the formation of cracks. It is possible to determine the maximum initial size of a microcrack which is about 10^{-4} cm[1], from the dependence of the gas pressure in a crack on its radius. Hence, the sizes of microdefects, in which the gas which cleaves the

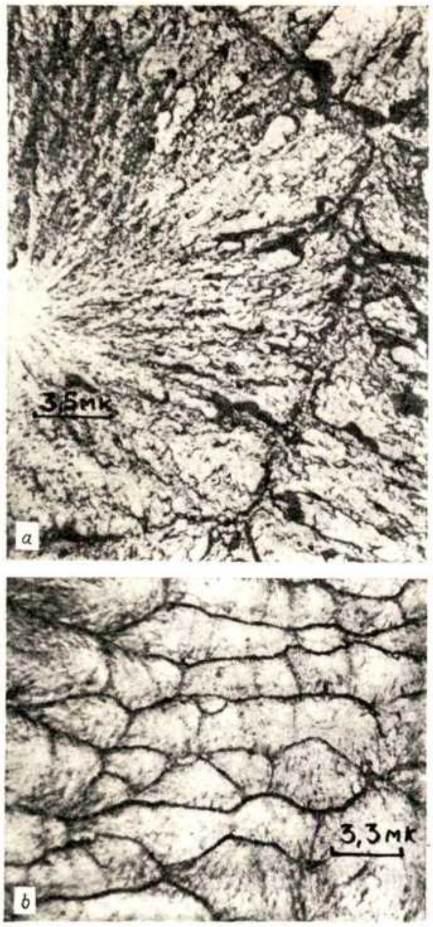

Fig. 1. Microstructure of block, amorphous PMMA (unirradiated sample). *a*, Large domain structure; *b*, small domain structure.

sample is formed, are not greater than 10^{-4} cm (PMMA and PS).

The location of microdefects can be determined by considering the relation between the microstructure and the destructions appearing in a polymer. The microstructures of amorphous polymers PMMA, PS and PC are essentially different. The microstructure of PMMA is characterized by domains, that of PS with batches of molecules rolled up into globules, and that of PC with batches of molecules combined into fibrillae located in the intersecting planes[4]. All of them, however, have one common feature—a great number of micropores (of linear dimensions up to 1 micron), which increase in number on stretching the sample[5]. PC has the greatest porosity. Fig. 1 shows the microstructure of two PMMA samples which differ in the domain diameters only. The corresponding destructions after the laser irradiation are shown in Fig. 2. The number of destructive cracks noticeably increases with a decrease in the average domain diameter. It may then be expected that the initiating microdefect nuclei are localized along the domain boundaries, where most of the PMMA micropores are situated. In equal conditions the number of destructions for PC is considerably greater than that for PMMA and PS (the porosity of polycarbonate is higher).

In the case of polycarbonate (PC) at 20° C, polymer structures become oriented in the vicinity of the developing cracks; this results in the localization of cracks. Because of this, the final size of the destructive cracks in PC is much less than that in PMMA or in PS, where there is no such localization. At −190° C localization does not occur, PC becomes fragile and its destruction differs from that of PMMA or PS only in the greater number of cracks present. Thus the sizes of the initiating microdefects coincide with those of the polymer micropores. They are located where most of the micropores are situated, and the number of destructions, in other words, the number of initiating microdefects, is higher the greater the number of micropores in the sample. We can therefore suppose that the microdefects are the micropores of polymer samples. This assumption can be checked by creating additional microdefect micropores in the sample by stretching it[5].

Table 1

σ (kg/cm^2)	0	100	200	300
n (cm^{-1})	11 ± 2	27 ± 3	36 ± 3	44 ± 6

Table 1 shows the value of the stretching stress σ and the number of destructive cracks per cm (n). These results indicate that the number of destructive cracks increases with the number of micropores (which is proportional to σ). The relation between σ and n is non-linear, because of the non-linear dependence of σ on the number of micropores appearing[5] and the random character of the process of crack formation under laser irradiation. Here, the external stretching force does not affect the mechanism of crack development and does not develop those cracks, where the gas pressure in the microdefects is not sufficient to make them visible. It is accounted for by the fact that the microcracks are of the order of 1 micron long so that they can be considered safe (unable to develop) at the values of σ listed in the table[6]. The stretching affects only the plane of orientation of the cracks and increases the initial volume of the opened micropores. Thus an increase in the number of micropores leads to an increase in the number of destructive cracks; in other words the destructive cracks are originated at polymer micropores.

Of course, not every micropore develops to the size of a destructive crack under the action of laser irradiation; some of them open up just slightly and can develop to visible sizes on subsequent irradiation[7]. It was proved experimentally[7] that during repeated irradiation destructive cracks appeared at those places where they were absent before, namely, at the points where micropores were opened by the previous laser pulses. The distribution of such opened micropores (as well as the destructive cracks) is not uniform, even in the same cross-section of the sample; this was proved by the method of light dispersion at these pores, and is probably due to the statistical

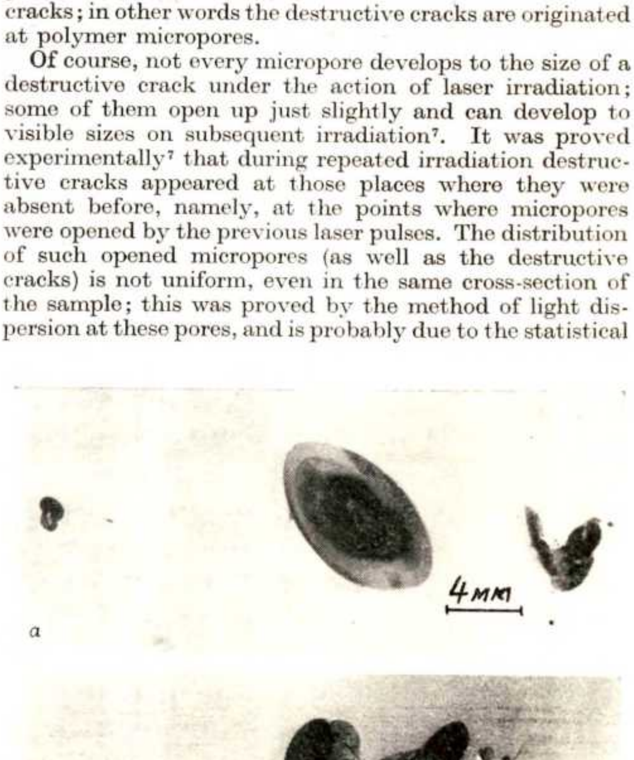

Fig. 2. Destructions in samples, subjected to laser irradiation, with large domain (*a*) and small domain (*b*) microstructure. Conditions in both cases are identical.

laws of crack formation under the effect of laser irradiation.

Thus all these experimental results suggest that the microdefect nuclei are micropores of materials such as PMMA, PS and PC.

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A "Dust Curtain" in Gaseous Core Nuclear Reactors for Rockets

The use of nuclear, rather than chemical, energy for the propulsion of space vehicles would greatly extend the possibilities of space travel, and in recent years much progress has been made towards the realization of nuclear propulsion devices. The principle of rocket propulsion rests on the production of a highly heated working fluid, called the propellant gas, which is expanded and ejected through a nozzle, thereby converting its thermal energy to directed kinetic energy. The heating of the propellant can be derived from the reaction of combustibles with a chemical system, or, in the nuclear system, from the nuclear reaction of fissile materials. In the nuclear reactor the fissile material is the fuel, and the thermal energy released by fission is transferred to the propellant, usually hydrogen. This energy transfer can be achieved by the use of a solid core which serves as an intermediate medium for the energy delivery to the propellant. Although the solid core reactor has reached a prototype engine demonstration after a decade of development[1], it does not, however, make the optimum use of the nuclear energy in a rocket engine. This stems from the fact that fission energy is released at very high level (10^6 eV or 10^{10} K) which has been degraded in a solid core reactor to 3,000 K, the limit of the operational temperature of a solid core heat exchanger. It is therefore desirable to use a gaseous core in which the fuel, namely the highly energetic fission particles, and the propellant are intimately mixed, thus transferring energy directly via collisions and radiation.

The difficulty of constructing a gaseous core reactor lies in providing a specific flow field for the mixture in the reactor cavity which fulfils the criticality condition for nuclear chain reaction and at the same time minimizes the loss of fuel to the exhaust and of energy to the cavity wall.

A novel concept of gaseous vortex reactor was proposed by Kerrebrock and Meghreblian[2] in which the gaseous mixture sustains a vortex flow which results in a peaked concentration of the fuel at an appropriate radial distance from the axis of a cylindrical cavity, with very small concentrations at the cavity wall and on the axis. In other words, the containment of the fuel, or fuel suspension in the cavity, is achieved by taking advantage of the balance between the differential centrifugal force and the diffusional drag of the fuel against the radially inward flowing propellant. Conceptually the propellant gas diffuses radially inwards through the hot, dense, annular cloud of fissioning fuel and then flows axially out through a nozzle in the centre of one end wall (see Fig. 1). Extensive theoretical and experimental studies have been made to determine the feasibility of this technique[3,4]. One of the limiting factors in the performance of a gaseous vortex reactor[2] or its modified versions[1] is the deposit of fission fragments, beta rays and thermal radiation on the cavity wall. This is compounded by the fact that at low temperatures ($\leq$7,000 K) hydrogen is relatively transparent to radiation. This loss is not only detrimental to fuel economy and the criticality for nuclear chain reaction of the engine, but also enhances the heating of the cavity wall. It is to reduce this loss that the concept of a "dust curtain" in the cavity of a gaseous vortex reactor is introduced.

It is recognized that seeding of the propellant with refractory metallic particles is very effective in increasing both its opacity to the radiation flux and its collision cross-section for the fission fragments. Because of its high molecular weight, the seeding material is, however, a poor substitute for the propellant from the viewpoint of optimum engine performance, and for this reason it would be desirable to have the seeding particles suspended and retained in the cavity, much like the fission particles, without being constantly ejected with the propellant through the exhaust nozzle. It is found that seeding particles, appropriately granulated in mass and size, which are premixed and taken in with the propellant in vortex flow, can be made to have a peaked concentration in an annular region at a specific radial distance from the axis of the cylindrical cavity. This annular seeding particle cloud is called here the "dust curtain", and is located between the surface of maximum fuel concentration and the wall to shield the latter from the bombardment and radiation of the fission particles.

Before formulating the desirable concentration distribution of the dust in the flowing propellant, it is necessary to consider the kinetics of small suspended particles in a non-uniform gas. The presence of the second admixture (the fission particles) in the propellant gas can be ignored, because the maximum concentrations of these two admixtures in the gas are in separate locations. Following the kinetic theories of Waldmann[5] and Mason and Chapman[6], the suspended dust particles are treated as giant molecules. The collisions between propellant gas molecules (mass m_p, density n_p, mass density $\rho_p = m_p n_p$), and the spherical dust particles (radius r_d, mass m_d,

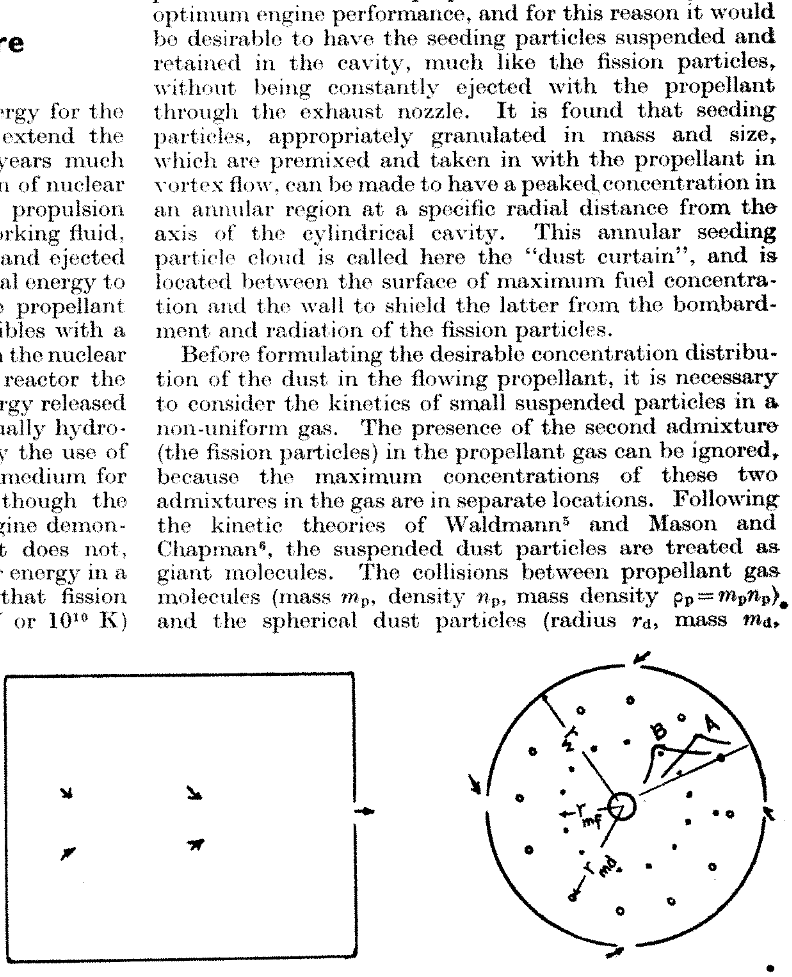

Fig. 1. Schematic gaseous vortex reactor. *A*, Distribution of ϱ_d/ϱ_p along r; B, distribution of ϱ_f/ϱ_p along r.

density n_d) are assumed to be elastic, a fraction α rebounding in random directions and the remainder specularly. The binary diffusion coefficient D_{dp} for such a quasi-Lorentzian gas can be expressed in the following manner[6]

$$\frac{1}{\rho_p D_{dp}} = \frac{8}{3}\left(\frac{2}{\pi m_p kT}\right)^{1/2}(\pi r_d^2)\left(1+\frac{4}{9}\alpha\right) \quad (1)$$

assuming $n_p \gg n_d$ and $m_d \gg m_p$.

Consider a binary mixture of inviscid gases, the propellant and the dust, in a steady, two-dimensional vortex flow of constant strength of vorticity $\Gamma = vr$, with a zero axial velocity in the annular region of interest and an inward radial velocity that is everywhere much smaller than the tangential velocity (see Fig. 1). In addition, the diffusion process is assumed to be non-turbulent, with negligible thermal diffusion and chemical reactions. The distribution of the density ratio of the gases ($w = \rho_d/\rho_p$) in the flow field can be formulated using the equations of continuity of the mixture flows and the diffusion velocity of the dust, following a similar mathematical deduction as in reference 2,

$$\frac{\dot M_p + \dot M_d}{\dot M_d} - \frac{1+w}{w} = \frac{2\pi \rho_d D_{dp}}{M_d}\left(\frac{d \ln w}{d \ln r} - \frac{m_d}{m_p}\frac{v^2}{RT}\right) \quad (2)$$

where R, T and $\dot M_p$ denote the gas constant (per unit mass), temperature and radial mass flow rate of the propellant gas respectively: $\dot M_d$, the radial mass flow rate of the dust. The value of $\dot M_d/\dot M_p$ is prescribed by the value of ρ_d/ρ_p at the inner boundary where the flow leaves the vortex; it gives the relative loss rate of seeding material and propellant. Equation (2) is completely analogous to the diffusion equation for the fission material in reference 2. If the dimensionless vortex flow v^2/RT is prescribed as a monotonically decreasing function of r, a simple maximum for $w(r)$ can be established with $w \to 0$ as $r \to \infty$. The position r_{md} of w_{max} may be determined by setting $dw/dr = 0$ in equation (2)

$$\frac{v^2}{RT} = \frac{\Gamma^2}{RTr_{md}^2} = - \frac{m_p}{m_d}\left(\frac{\dot M_p + \dot M_d}{2\pi \rho_p D_{pd}}\right)\left[1 - \left(\frac{1+w_m}{w_m}\right)\left(\frac{\dot M_d}{\dot M_p + \dot M_d}\right)\right] \quad (3)$$

An effective shielding of the wall (r_w) can be attained by setting $r_{mf} < r_{md} < r_w$ where r_{mf} denotes the location of the maximum concentration of the fuel. A desirable value of r_{md} can be obtained by a judicious choice of size and mass of dust particles relative to those of the fission particles and also those of the propellant gas (see equation (3)).

This work was initiated while I was a consultant to the Vehicle Propulsion Division of NASA Office of Space Science and Applications. I thank Dr R. B. Morrison for his interest and encouragement, and Mr Keith Bekofske who helped in the calculations to check the feasibility of the idea.

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Effects of Shear Treatment on Drag-reducing Polymer Solutions and Fibre Suspensions

NUMEROUS workers have put forward models which attempt to explain the complex behaviour of drag reducing polymer solutions, but no explanation has been universally accepted. The ability of apparently identical batches of a polymer to give widely varying results has now been ascribed to variations in mixing procedures, though apparently no systematic investigation of the reason for such behaviour has been made. In the experiments reported here, concerning drag reduction in 'Polyox *WSR* 301' solutions and asbestos fibre dispersions, attention is focused on the differences in behaviour in large and small bore pipes and the changes in this behaviour brought about by shear thinning treatment.

Tubes of 0·115 cm bore (70 cm length) and 1·43 cm bore (300 cm length) were used, at a Reynolds number of approximately 2×10^4 in each case. 'Polyox' solutions were prepared by adding water quickly and in bulk to a dispersion of 'Polyox' powder in glycol. The asbestos fibres, kept in a surfactant dispersion, were specially prepared by Turner Bros Asbestos Co. Ltd to maintain a high length to diameter ratio. Surfactant solution was used in diluting the asbestos, and the asbestos drag measurements were referred to the drag of the surfactant solution, though in all cases the surfactant gave drag figures identical with water.

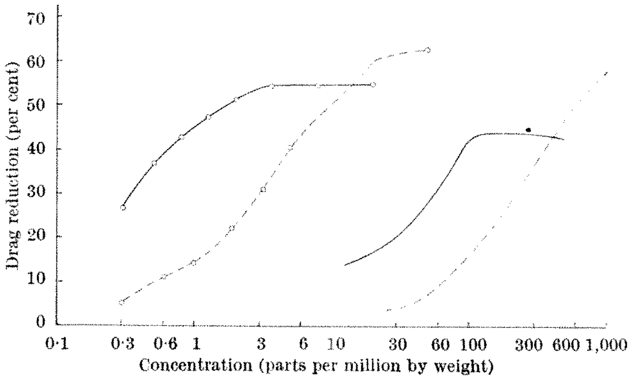

Fig. 1. Drag reduction versus concentration, 'Polyox *WSR* 301' and asbestos fibre suspensions. 'Polyox *WSR* 301': ○—○, 1·43 cm tube; ○ - - - ○, 0·115 cm tube. Asbestos: ———, 1·43 cm tube; - - -, 0·115 cm tube.

Fig. 1 shows drag reduction as a function of concentration in both tubes, for 'Polyox' and asbestos. This is believed to be the first time that high values of drag reduction (44 per cent) have been obtained with dilute fibre dispersions (100 parts per million by weight).

Table 1. DRAG REDUCTION WITH 'POLYOX *WSR* 301': EFFECTS OF SLIGHT SHEAR THINNING

'Polyox' concentration (parts per million by weight) (w.p.p.m.)	Drag reduction 1·43 cm diameter tube (per cent)	
	First pass	Second pass
0·64	40	26
0·32	27	13

Table 1 shows the degradation in performance obtained when 'Polyox' solutions were passed through the 1·43 cm tube, collected and passed through again. A similar experiment was carried out on asbestos dispersions, but no change was observed in this case. It appears that even the slight shear thinning applied during the first pass has markedly reduced the polymer effectiveness in this situation.

To carry the investigation further, quantities of the original 0·1 per cent 'Polyox' solution and 0·1 per cent asbestos dispersion were subjected to shear treatment for one hour by recirculation through an impeller pump, and

Table 2. DRAG REDUCTION WITH 'POLYOX *WSR* 301' AND ASBESTOS; EFFECTS OF PROLONGED SHEAR THINNING

Pipe diameter	Condition	'Polyox *WSR* 301' (10 w.p.p.m.) per cent	Asbestos fibres (50 w.p.p.m.) per cent
0·115 cm	Unpumped	51	49
	Pumped	50	42
1·43 cm	Unpumped	54	44
	Pumped	1	33

were then tested in both tubes. The results, compared in Table 2 with results obtained for unpumped samples, demonstrate a remarkable change in behaviour of 'Polyox'; whereas the drag reduction figure in the small pipe changed negligibly with pumping, in the large pipe it has dropped from 54 per cent to zero, within experimental error. The effectiveness of asbestos, on the other hand, has only dropped slightly in both tubes. The pumped 'Polyox' was also tested as a function of concentration in the small tube, but the resultant graph was so close to the result shown in Fig. 1 as to be indistinguishable.

These results demonstrate that drag reduction with polymers in turbulent pipe flow is dependent on the shear history of the fluid only when the pipe is comparatively large. It is my opinion, however, that it is the scale of turbulence, and not simply pipe size, which is the parameter governing this effect.

Of the numerous physical models which have been put forward in an attempt to explain all the properties of drag reducing polymers, that proposed by Alan White[1] has hitherto appeared the most reasonable. White supposes a dual action by the polymer; a thickening of the laminar sublayer, which occurs whether the polymer is "fresh" or "shear treated", and a turbulence damping effect, which occurs only when the polymer is not shear treated or aged.

The present results suggest that for a full description of drag reduction, one needs to specify some parameter which is linked to the pre-treatment of the polymer; the "stringiness" at high concentration, in other words the tendency to form thread-like filaments, is an obvious candidate. The results may be explained by assuming that the stringiness of, say, a 0·1 per cent 'Polyox' solution is linked to the size of energy absorption domains (presumably consisting of matrices or agglomerates of molecules containing large entrapped volumes of water) which interact in some way with flow instabilities to inhibit their growth.

If the permanent thinning of polymer solutions by the application of shear were simply a reduction in domain dimensions, one would expect no decrease in effectiveness in situations where the reduced domain size (down to molecular dimensions) was still adequate to interact with the largest energy dissipating vortices encountered (small tube, high wall shear stress), but a greatly reduced drag reducing effect in situations where the scale of turbulence was much larger (large tube, low wall shear stress). The results described in this report exhibit precisely this behaviour, and it is suggested as a consequence that polymer drag reduction should again be regarded as a "single-action" phenomenon; that of instability damping, where the physical scale of turbulence and a stringiness type parameter are important factors.

The results obtained for asbestos, while being interesting in themselves, tend to reinforce this model. Presumably the individual asbestos fibres are large enough to affect the largest turbulence scale in the large tube, and the smaller observed change in drag reduction on pumping is probably due to a degree of flocculation or scission brought about by pumping. Asbestos fibres may be found to lose their drag reducing properties in turn if the turbulence scale is pushed high enough.

It seems probable that organic polymers have little future in recirculatory pumping systems, for they lose their invaluable large domain size/turbulence damping properties at low values of shear stress. Ionic soaps akin to cetramide may repay study, but fibres will certainly repay further investigation, for they seem to be on the right scale of dimensions for drag reduction in large scale turbulence. They are also more tractable than polymers, being much larger, and could make the basic understanding of drag reduction an easier problem to study.

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Energy Resonances between Molecular Species in CO_2 Gas Lasers

IMPORTANT requirements for the attainment of population inversion needed in the operation of molecular gas laser systems are that population of the upper laser level and depopulation of the lower level(s) should be attained as far as possible through super-elastic collisions with impurity species[1]. These and other conditions[1,2] are well satisfied by CO_2 lasers operating in a number of gas mixtures[3] such as CO_2, CO_2-N_2, CO_2-N_2-H_2O, CO_2-N_2-H_2O-He. For example, it is well known[4] that for lasers operating in CO_2-N_2 mixtures, the close energy resonance between the metastable $N_2(X^1\Sigma_g^+)$ and the upper laser level $CO_2(X^1\Sigma_g^+, 0, 0^\circ, 1)$ provides a very efficient super-elastic collision channel for selective population of this CO_2 level. It is also possible that other low-lying vibrational levels of $N_2(X^1\Sigma^+)$ may play similar parts.

It is the purpose of this report to draw attention to a number of other close energy resonances which exist between laser levels of CO_2, and the diatomic and triatomic species expected to be present in various discharges in which CO_2 laser action has been achieved. CO_2 laser action in the 9·6 micron and 10·6 micron bands is achieved in a variety of discharges through pure CO_2 (and additives) at a few millimetres pressure. Efficiency is increased by addition of N_2, H_2O and He[3]. Efficient CO_2 laser action has recently been reported at atmospheric pressure, excited by transverse high voltage discharges[5].

Varying amounts of the following species will be expected to be present in the laser discharges depending on excitation conditions

$CO_2, CO_2^+, CO, CO^+, O_2, O_2^+, C_2, C_2^+$

$N_2, N_2^+, NO, NO^+, CN, CN^+$

$H_2O, H_2O^+, OH, OH^+, CH, CH^+, NH, NH^+$

There is optical and mass spectrographic evidence for most of these. For example, the CO_2^+ bands are the major contributor to the optical spectrum of discharged CO_2. Before a quantitative examination can be made of the excitation kinetics of CO_2 laser levels, energy resonances between them and levels of the above species must be examined because high excitation cross-sections will be expected in super-elastic collisions which involve close energy resonances. The energy range (0–2,349 cm^{-1}) of interest implies that only low-lying ($v = 1$) vibrational levels of the electronic ground state of species need be considered here. Further, most CO_2 lasers operate at gas temperatures of about 400 K[3], which implies rotational development of 300 cm^{-1} of each vibrational level. Thus in the review of energy resonances made here, each vibrational level is treated as an energy band of about 300 cm^{-1} width. The relative locations of the energy levels of the species listed above are compared in Fig. 1 with the location of the CO_2 laser levels. For ease of comparison, resonant levels are separated from non-resonant levels in this figure. Data for diatomic species were taken from Wallace's compilation[6], while data for polyatomic species were taken from Herzberg's compilation[7]. No data were available for C_2^+ or H_2O^+. The ground state of C_2 was called a $^1\Sigma$ by Wallace[6].

It is clear from Fig. 1 that, in addition to the strong resonance between the upper laser level $CO_2(0,0^\circ,1)$ and $N_2(X^1\Sigma_g^+, v=1)$ to which reference has already been made, a number of other strong and partial resonances exist. In particular, the stable ion NO^+, which plays important parts in ionospheric physics, exhibits a somewhat stronger energy resonance than N_2 with the upper laser level $CO_2(0,0^\circ,1)$. CO, CO^+ and N_2^+ also exhibit strong energy resonances with the upper laser level. They can all thus play parts in maintaining the population inversion. Vibrational levels of N_2^+, like those of N_2, are metastable because both molecules are homonuclear. It is also noted that the (2,0,0) level of CO_2^+ is in partial resonance with $CO_2(0,0^\circ,1)$. It is also seen in Fig. 1 that the two lower CO_2 laser levels, $(0,2^\circ,0)$ and $(1,0^\circ,0)$, are in resonance with CO_2^+ (1,0,0 and 0,0,1), with H_2O $(0,1^\circ,0)$ and with O_2. Because of its metastability and its relatively long life, $O_2(v=1)$ probably contributes to the collisional population of the lower laser levels, although O_2 is easily dissociated by energetic electron collision through the operation of the Franck-Condon principle. It may thus not be present in large quantities.

Witteman[9] has shown that H_2O resonances increase the depopulation of the lower laser level. Excited CO_2 transfers its energy to unexcited H_2O in super-elastic collisions. The resulting excited H_2O is strongly collisionally de-excited by other H_2O molecules. It is thus not unlikely that the ground state CO_2^+ molecules can similarly act to depopulate the lower laser levels of CO_2. The (2,0,0) CO_2^+ level may similarly act to depopulate the upper laser level of CO_2. The energy resonance between rotational structures in this case is not so large, however, and decreases with decrease in temperature. The energy resonance between the lower CO_2 and CO_2^+ levels is contrastingly larger, and thus retained even at lower (200 K) temperatures. Thus the balance between CO_2^+ depopulation of upper and lower levels moves towards increasing population inversion with decreasing temperature. This effect is observed experimentally[3]. Species arising from CO_2 alone must in all probability be responsible for some population inversions in pure CO_2 lasers. The possible effects of ionic species like CO_2^+ and NO^+ cannot be overlooked in the operation of lasers operating in many discharges, including those maintained by high voltage excitation at atmospheric pressure. Relatively little work has been done on the kinetics of excitation of laser levels[9,10] and the resonances described in this report do not seem so far to have been considered.

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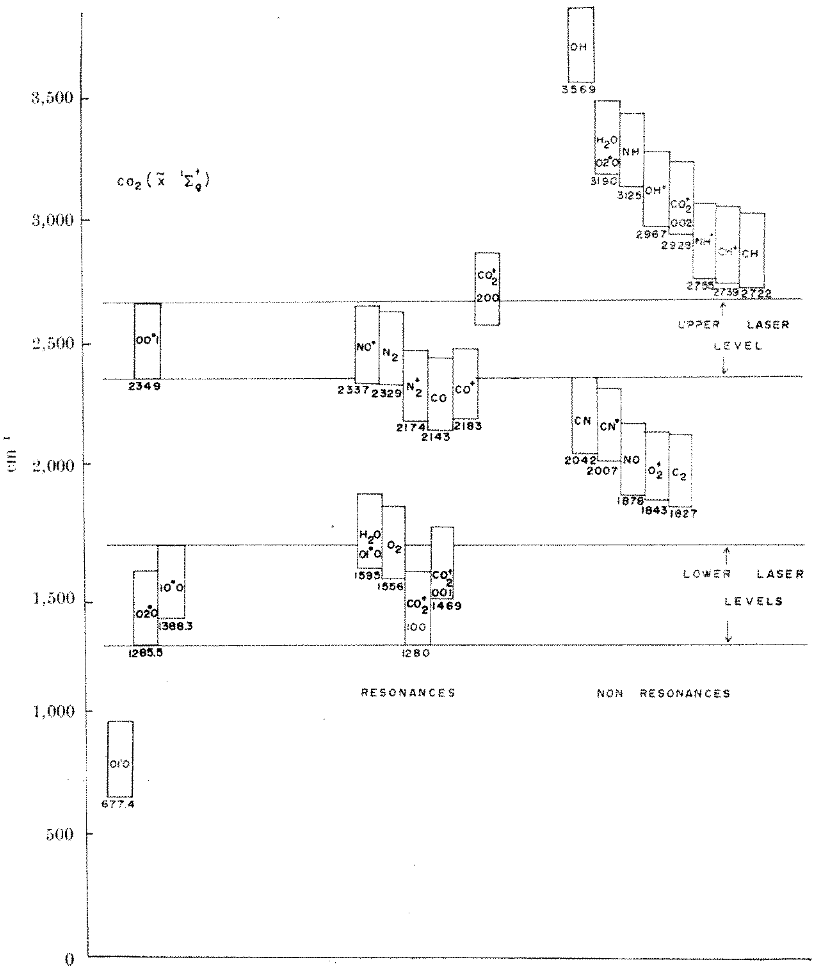

Fig. 1. Energy resonances between vibrational levels of molecular species in CO_2 gas lasers. Note that each level is broadened by about 300 cm⁻¹ equivalent to a gas temperature of about 400 K.

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BIOLOGICAL SCIENCES

Haemoglobin α Chain Duplication in Barbary Sheep, *Ammotragus lervia*, *Pallas, 1777*

EVIDENCE for the existence of two non-allelic Hb_α structural loci in the domestic goat has been presented[1,2]. The products of these loci, termed $^I\alpha$ and $^{II}\alpha$ chains[3], differ in at least four positions. Glycine, alanine, leucine and asparagine occupy positions 19, 26, 113 and 115 in the $^I\alpha$ chain, respectively, whereas serine, threonine, histidine and serine are present in these positions of the $^{II}\alpha$ chain[2]. The percentages of $^I\alpha$ and $^{II}\alpha$ chains in red cell haemolysates

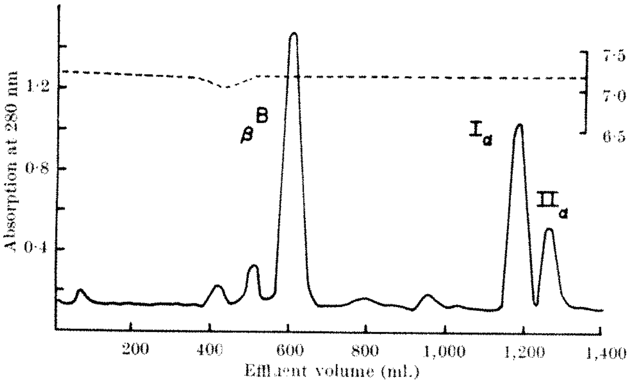

Fig. 1. Separation of the α and β chains of haemoglobin from Barbary sheep No. 503 by CM-cellulose chromatography. Globin (280 mg) was used as starting material.

of normal AA goats are about 72 and 28, indicating that evolution of the duplicated Hb_{α} loci is associated with a three-fold decrease (or increase) in Hb_{α} locus efficiency[3]. No such α chain multiplicity was observed in the domestic sheep[4].

We describe here similar observations for the α chains of the haemoglobin from Barbary sheep (*Ammotragus lervia, Pallas, 1777*). A distinct haemoglobin heterogeneity was found in this animal species[5-7]; the β chain of one of these haemoglobins was recently characterized[7].

Material was available from two animals; one (numbered 8) which was homozygous for haemoglobin type C was studied previously[7], while a second (numbered 503) with a homozygosity for Hb-B was obtained commercially. The α and β^{C} chains from globin of animal numbered 8 were separated by countercurrent distribution[7], while the globin from animal numbered 503 was analysed by CM-cellulose chromatography[3]. Fig. 1 illustrates the separation of the haemoglobin chains by chromatography; one major β chain (the β^{B} chain) was eluted first, followed by two α chains, which were completely separated from each other. The relative amounts of the $^{\rm II}\alpha$ zone were 25·7, 24·0 and 24·8 per cent in triplicate analyses, indicating that the $^{\rm I}\alpha$ and $^{\rm II}\alpha$ chains in this animal are present in a ratio of about 3 to 1, similarly as observed in the normal AA goat[3].

The $^{\rm I}\alpha$ and $^{\rm II}\alpha$ chains were isolated from the respective zones of the three chromatograms by passage through 60 × 2·5 cm columns of 'Sephadex *G*-25' in 0·5 per cent formic acid, and subsequent freeze drying of the protein containing zones. Structural analyses were limited to the determination of the amino-acid compositions of the tryptic peptides, and of the chymotryptic peptides from the insoluble cores. The techniques are the same as those used in the study of goat and sheep α chains[2,4].

No differences were observed between the amino-acid compositions of the tryptic peptides T-1; T-2; T-3; T-5; T-6; T-7,8; T-9^{a}; T-10; T-11; T-10,11 and T-14 of the $^{\rm I}\alpha$ chain and those of the same peptides of the $^{\rm II}\alpha$ chain. Nor do the amino-acid compositions of these peptides differ from the compositions of the corresponding peptides of goat α chains[2]. The chymotryptic peptides, originating from positions 99 through 106 and from positions 118 through 141 of the cores from both α chains, also had identical amino-acid compositions, which again did not differ from those of the corresponding goat α chain core peptides.

The compositions of the tryptic peptides T-4 and T-9^{b} and of a chymotryptic core peptide from both chains are presented in Table 1. Comparison of these data with those observed for the corresponding peptides of the goat $^{\rm I}\alpha$ and $^{\rm II}\alpha$ chains[2] indicates: (*a*) the amino-acid compositions of the peptides T-4 and core C-2 of the $^{\rm I}\alpha$ chain are identical to those of the goat $^{\rm I}\alpha$ chain; (*b*) the amino-acid compositions of peptides T-4 and core C-1 of the $^{\rm II}\alpha$ chain also do not differ from the corresponding peptides of the goat $^{\rm II}\alpha$ chain, except for the presence of one more residue of threonine and one less residue of alanine in the T-4 peptide of the goat $^{\rm II}\alpha$ chain; (*c*) the T-9^{b} peptides from both chains have identical amino-acid compositions, but differ from the T-9^{b} peptide of the goat α chains by the presence of one less residue of aspartic acid, which is replaced by glutamic acid. Similar analyses made on the mixture of the two α chains from Barbary sheep numbered 8 gave comparable results. When the amino-acid residues of the two T-4 peptides and of the core peptides are placed in sequence by analogy with known sequences of goat and sheep α chains[2,4], three differences between the two α chains of Barbary sheep haemoglobin are apparent. Positions 19, 113 and 115 of the $^{\rm I}\alpha$ chain are occupied by glycine, leucine and asparagine, respectively, whereas serine, histidine and serine are present in these positions of the $^{\rm II}\alpha$ chain. An alanyl residue likely occupies position 26 of both chains (alanine in goat $^{\rm I}\alpha$ chain and threonine in goat $^{\rm II}\alpha$ chain).

Table 1. AMINO-ACID COMPOSITIONS OF SOME TRYPTIC AND CHYMOTRYPTIC PEPTIDES OF THE $^{\rm I}\alpha$ AND $^{\rm II}\alpha$ CHAINS FROM THE HAEMOGLOBIN OF BARBARY SHEEP NUMBERED 503

Amino-acid	αT-4 $^{\rm I}\alpha$	αT-4 $^{\rm II}\alpha$	αT-9^{b} $^{\rm I}\alpha$	αT-9^{b} $^{\rm II}\alpha$	Core C-2 $^{\rm I}\alpha$	Core C-1 $^{\rm II}\alpha$
Lys			1·00 (1)	1·00 (1)		
His			2·84 (3)	2·60 (3)	2·00 (2)	2·00 (2)
Arg	1·00 (1)	1·00 (1)				
Cyst. acid					0·93 (1)	0·87 (1)
Asp	1·05 (1)	1·12 (1)	2·89 (4)	2·81 (4)	2·11 (2)	1·12 (1)
Thr		(1)	1·00 (1)	1·14 (1)	1·99 (2)	1·06 (1)
Ser		1·09 (1)	1·86 (2)	1·87 (2)		1·06 (1)
Glu	2·03 (2)	1·77 (2)	0·98 (0)	1·00 (0)		
Pro			0·98 (1)	1·00 (1)	2·07 (2)	0·98 (1)
Gly	3·98 (4)	2·84 (3)	1·96 (2)	1·91 (2)		
Ala	3·98 (4)	3·86 (3)	1·85 (2)	2·08 (2)	2·03 (2)	1·03 (1)
Val	0·95 (1)	0·97 (1)	0·91 (1)	1·05 (1)	1·92 (2)	0·85 (1)
Leu	1·02 (1)	1·07 (1)	5·17 (5)	4·72 (5)	2·16 (2)	1·17 (1)
Tyr	0·93 (1)	0·91 (1)				
Phe					0·99 (1)	0·90 (1)
Positions in chain	17–31	17–31	69–90	69–90	107–122	107–117

* Data are presented as numbers of residues relative to lysine, arginine or histidine. The nomenclature used is the same as for the peptides from goat α chains[2]. Numbers between parentheses denote amino-acid compositions of corresponding goat α chain peptides.

These findings, although observed in only two animals, indicate a chemical heterogeneity of haemoglobin from Barbary sheep, which is closely comparable with that of the haemoglobin from the domestic goat. It therefore seems reasonable to assume that this heterogeneity results from a similar genetic factor, namely duplication of the Hb_{α} locus and subsequent evolution of the resulting $^{\rm I}\alpha$ and/or $^{\rm II}\alpha$ loci. Our observations add another animal species to the growing list of mammals in which multiplicity of Hb_{α} loci has been demonstrated (summarized in ref. 3). The similarity of the differences between the $^{\rm I}\alpha$ and $^{\rm II}\alpha$ chains from Barbary sheep and of those between the $^{\rm I}\alpha$ and $^{\rm II}\alpha$ chains from the goat supports the hypothesis that species *Ammotragus lervia, Pallas, 1777*, should be placed in the genus *Capra* rather than in the genus *Ovis*, as is most frequently the case[8].

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In vivo Testing of the Fletcher–Huehns Hypothesis of Functional Differences of Iron Atoms bound by Transferrin

RECENTLY Fletcher and Huehns[1-3] proposed an interesting hypothesis on the possible functional difference between the two iron binding sites of the iron transport protein, transferrin. They presented evidence from *in vitro* studies that iron uptake by the two binding sites is random, while the release of iron to erythroid precursors occurs preferentially from one site, which they arbitrarily designated the A site. This site is also postulated to be important in the transfer of iron to the placenta of pregnant animals. The other site, designated B, is somehow concerned with delivery of iron to storage depots. Complete saturation of both sites with iron produces greater transfer to erythroid precursors and/or storage depots. The concept of selective release of iron from functionally different binding sites on the transferrin molecule is potentially of great importance and warranted repetition of Fletcher and Huehns's *in vitro* experiments and the exploration of their hypothesis *in vivo*.

The procedures for collecting blood enriched with reticulocytes and incubating them with labelled transferrin were those described originally by Jandl and co-workers[4,5] and modified by Kornfeld[6]. Purified, iron-free, human transferrin (Behringwerke) was used in all experiments.

Transferrin, 100 per cent saturated with ^{59}Fe, was preincubated with rabbit reticulocytes until 30 to 50 per cent of the ^{59}Fe was removed from solution. The transferrin solution was freed of reticulocytes by centrifugation and then incubated with fresh reticulocytes for 60 min at 37° C. The uptake of iron from the predominantly B site binding of this transferrin solution was diminished by 70 per cent compared with the uptake from a control preparation of ^{59}Fe-transferrin of equal saturation that had ^{59}Fe bound randomly to A and B sites. Furthermore, when ^{59}Fe was added back to the preincubated transferrin to restore 100 per cent saturation, ^{59}Fe uptake by fresh reticulocytes was restored to the control levels, confirming the original observations of Fletcher and Huehns.

To test *in vivo* the hypothesis of selective iron distribution from the different binding sites of transferrin, double-labelled transferrin was prepared. Transferrin was totally saturated with ^{59}Fe and then incubated with rabbit reticulocytes until 30 to 50 per cent of the ^{59}Fe had been removed from the transferrin solution (presumably most of the ^{59}Fe was removed from site A). Complete saturation was restored by adding ^{55}Fe to achieve selective labelling of site A while site B retained its original ^{59}Fe. Alternatively, the transferrin was first saturated with ^{55}Fe and the ^{59}Fe was added to replace the iron removed by incubation with reticulocytes. The transferrin solutions with selective tagging of A and B sites were injected intravenously into rats. The 2 h uptake of radioactivity, expressed as a percentage of the dose administered, was calculated for selected organs which were analysed for ^{55}Fe and ^{59}Fe simultaneously by the method of Katz and co-workers[7]. In seven experiments, pregnant rats were studied for evidence of selective placental radio iron transfer (Table 1).

A significant difference in the tissue distribution of radioactive iron was observed in only two instances in these rats injected *in vivo*. Selective labelling of site B produced enhanced uptake by the foetuses of pregnant rats which is contrary to the prediction of the Fletcher–Huehns hypothesis. Diminished radio iron in the blood of rats injected with site A labelled transferrin in comparison with uniformly labelled transferrin but not with site B labelled transferrin seems to have statistical rather than biological significance. Most noteworthy is the lack of selective uptake of radio iron of a specific type by bone marrow, liver or spleen, receptor tissues with expected polarity for selectively labelled transferrin.

We conclude from these studies, which were designed to test *in vivo* the release of iron from selectively labelled transferrin, that the differences of iron transfer predicted by *in vitro* studies are not demonstrated and that a re-examination of Fletcher and Huehns's attractive hypothesis is necessary.

Table 1. PERCENTAGE UPTAKE OF RADIO IRON (MEAN ± SD)

Tissue	Equal labelling of transferrin sites A and B (9)	Predominant labelling of transferrin site B (20)	Predominant labelling of transferrin site A (13)	Significance Student's *t* test
Bone marrow	44·1 ± 10·1	44·6 ± 18·1	47·8 ± 9·7	N.S.
Blood	16·2* ± 1·1	15·4 ± 12·4	11·4* ± 5·1	0·05 > *t* > 0·025
Liver	18·2 ± 6·3	16·9 ± 12·0	14·2 ± 7·1	N.S.
Spleen	2·2 ± 0·6	3·4 ± 2·6	3·3 ± 2·7	N.S.
Maternal placenta		(4) 0·2 ± 0·16	(3) 0·2 ± 0·13	N.S.
Foetal placenta		1·9 ± 0·81	1·9 ± 0·46	N.S.
Foetus		2·7* ± 2·09	0·91* ± 0·48	0·05 > *t* > 0·025

Rats (250–380 g) were injected intravenously with transferrin totally labelled with radio iron on selected iron-binding sites. Tissue distribution of ^{59}Fe and ^{55}Fe expressed as per cent of injected radioactivity was measured at 2 h. The number of experiments is given in parentheses and significant differences are shown by *. Bone marrow mass is calculated as 2 per cent of body weight, blood volume as 5 per cent of body weight. Four to ten foetuses in the last third of gestation were analysed for each pregnant rat.

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Crystalline Fc prepared in High Yield from Normal Human IgG

CRYSTALLINE rabbit Fc fragment is simple to prepare in high yield from the corresponding IgG by cysteine-activated papain digestion[1]. Although crystalline products can be obtained from human myeloma globulins, only one report[2] has hitherto appeared describing an analogous substance from normal human IgG. The conditions for digestion of normal rabbit or human IgG were markedly different. One-tenth the ratio of enzyme: antibody was used, and the reaction time was reduced from 16 h to 30 min for the human material. In our hands, although a solid human Fc was obtained by the method of Hershgold *et al.*, the material did not crystallize, and the yield was low. We therefore decided to re-examine the cysteine-activated digestion conditions. The results are reported here, together with the finding that the crystalline Fc, obtained in high yield, may be principally of a single Gm allotype, reflecting the allotypy of the IgG 1.

Human IgG was prepared from the serum of single Caucasian individuals by DEAE-cellulose chromatography[3], and checked for purity by immunoelectrophoresis. In preliminary experiments, an IgG which contained an anti-D rhesus agglutinin was used to indicate approximately the conditions necessary for cleavage. Globulin was incubated with 1 per cent of its weight of crystalline papain (Worthington) in 10 mM cysteine and 2 mM EDTA at 37° and *p*H 7. Although agglutination was abolished within 1·5 h, the globulin immunophoretic arc had not competely disappeared. These conditions were, nevertheless, selected for studies on a larger scale.

Incubation was followed by dialysis against 10 mM sodium phosphate (*p*H 7·6) at 4° for 6 h, and chromatography of the digestion mixture on carboxymethyl-cellulose as described by Franklin[4]. This resulted in two well separated peaks, the first containing Fc and the second containing F(ab). Both peaks contained undigested IgG. The F(ab) (molecular weight about 50,000: from calibrated 'Sephadex *G*-200' columns[5]), when derived from an anti-D agglutinin, was the only digestion fragment to exhibit blocking of the original IgG by previous incubation with target cells.

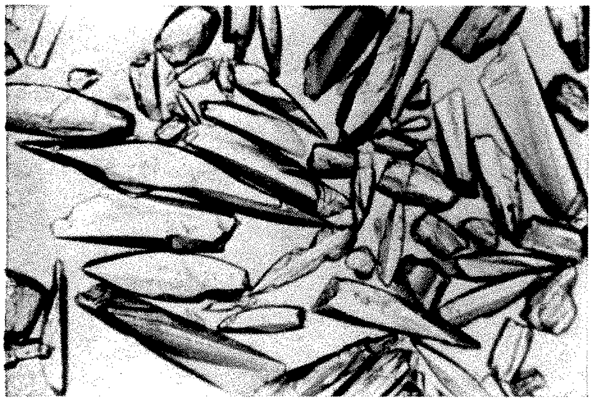

Fig. 1. Crystalline Fc from normal human IgG by papain digestion. × 20.

Upon concentration of peak 1 to a small volume by membrane filtration, a large yield of crystals was obtained (Fig. 1). Chromatography on 'Sephadex *G*-200' of the mother liquors after removal of crystals by centrifugation is illustrated in Fig. 2*a*, from which it can be seen that IgG is also present, confirmed by double diffusion on agar and immunophoresis. The relative amounts of Fc and IgG are not deducible from this figure, because much of the Fc has been removed by centrifugation. Recrystallization was easily effected from 200 mM phosphate *p*H 7·6, by dialysis at 4° against 2 mM phosphate buffer. Chromatography of the product after three recrystallizations is illustrated in Fig. 2*b*. The molecular weight of the crystalline product is about 50,000, and it gives a single line on immunoelectrophoresis. The yield of crystals is frequently over 60 per cent of the theoretical, based on the IgG actually destroyed. Preliminary allotyping data are given in Table 1.

Table 1. ALLOTYPING OF IgG AND CRYSTALLINE Fc FRAGMENT

Sample	Allotype Gm (1)	Gm (5)	Gm (21)
D IgG	−	+	−
D Fc	−	(±)*	−
L IgG	+	+	+
L Fc	+	−	−

* Fc present at 8 mg/ml.; Gm(5) present < 0·25 mg/ml.

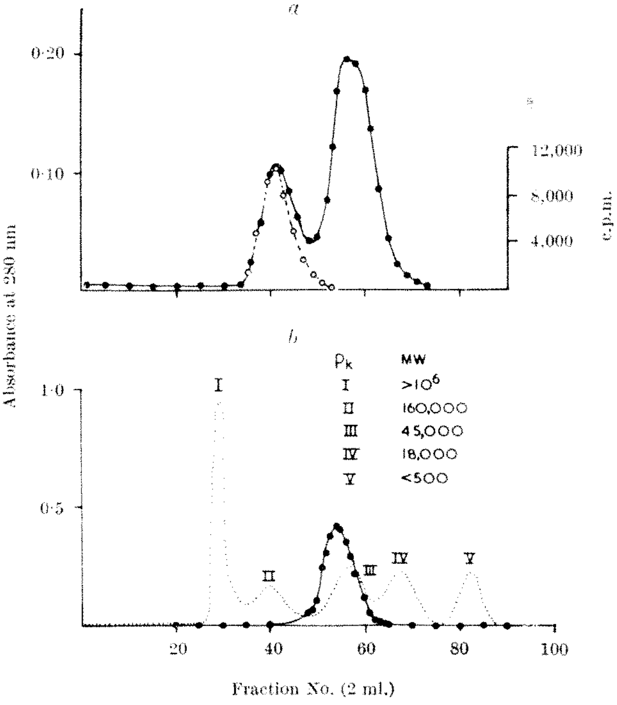

Fig. 2. *a*, 'Sephadex *G*-200' chromatography in phosphate-buffered saline of mother-liquors of "Fc" peak from carboxymethyl-cellulose[4], after removal of crystals. ^{125}I-IgG was included in column influent solution. ●—●, $A_{280\ \mathrm{nm}}$; ○ - - - ○, radioactivity (c.p.m.). *b*, 'Sephadex *G*-200' chromatography in phosphate-buffered saline of a solution of thrice recrystallized Fc. ●—●, $A_{280\ \mathrm{nm}}$; . . . . , standards used in column (2 × 90 cm) calibration.

Of the four classes of human IgG, IgG 3 is the most sensitive to papain, followed by IgG 1, and then IgG 2 and IgG 4 which have much lower sensitivities. It is clear that of the total crystalline product, less than 3 per cent is derived from IgG 3 (the Gm 5 and Gm 21 markers), and therefore molecules bearing Gm 1 or non-Gm 1 account for most of the crystalline protein in the examples described. IgG 2 and IgG 4 contribute only 14 per cent[6] of the total IgG in serum, and cannot therefore explain the yield. Until more data are obtained from other allotypes, and the undigested IgG is examined, it cannot be established whether co-crystallization or mixed crystals have resulted in contamination of the Fc allotype(s) obtained from IgG 1. Such contamination must, however, be small. This ignores the possibility that the L IgG individual examined could well be heterozygous for Gm 1, and the crystals could be 50 per cent non-Gm 1.

From the results of recent work[7,8], it seems that the Gm 1 and non-Gm 1 specificities of IgG 1 subclass are sensitive to prolonged papain digestion. Pepsin-produced pFc′ fragments retain the Gm allotypy of their respective Fc molecules, but on incubation with papain, or by exhaustive incubation of IgG with papain, such markers are lost. Our incubation conditions fortuitously represent circumstances in which papain-sensitive bonds in IgG 1 molecules which connect Fc to Fd are ruptured quantitatively, and yet further breakdown to Fc′ is slight. Fc derived from IgG 3 is either removed during recrystallization or principally converted to Fc′ during 1·5 h incubation with papain. This arc may have been overlooked in our immunoelectrophoresis patterns.

It will be useful to have normal human IgG more easily available than paraproteins for X-ray crystallography. Moreover, studies on crystalline fragments of a single IgG 1 allotype among families may be very informative about the molecular nature of their isotypic specificity.

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Use of Phenylalanyl-tRNA–Guanosine-5′-Triphosphate–TI$_u$ Complex in Peptide Bond Formation

IT has been demonstrated[1-10] that in bacterial systems a complex, designated complex II, that is composed of aminoacyl-tRNA, GTP, and the heat labile transfer factor TI$_u$* serves as an intermediate in the binding of aminoacyl-tRNA to ribosomes and in the elongation of polypeptide chains. When Phe-tRNA from complex II is bound to a ribosome-poly U complex carrying AcPhe-tRNA at the donor site, most of the Phe-tRNA bound to the ribosomes interacts with the AcPhe-tRNA giving rise to AcPhe-Phe-tRNA at the acceptor site[6,10,12,13]. Phe-tRNA from complex II is also bound at the acceptor site of a ribosome–poly U complex having an unoccupied donor site, but in this case very little dipeptide is formed[9,10]. In both instances, approximately one GTP from complex II is cleaved for each Phe-tRNA bound to the ribosomes[9,10,14,15]. Lack of dipeptide formation is also observed in the presence of the antibiotic, sparsomycin[6,13,16], or when the GTP analogue 5′-guanylmethylene diphosphonate is substituted for GTP[12,13]. Our results indicate that almost none of the Phe-tRNA from complex II bound to the acceptor sites before the addition of AcPhe-tRNA to donor sites is incorporated in peptide chain.

AcPhe-tRNA bound to ribosomes at 5 mM Mg^{2+} in the presence of GTP and initiation factors reacts almost quantitatively with puromycin (Table 1), indicating that it is bound at the donor sites of the ribosomes. The binding of Phe-tRNA from complex II to these ribosomes results in a decrease in the amount of AcPhe-tRNA that reacts with puromycin, indicating the formation of AcPhe-Phe-tRNA at the acceptor sites. Identification of the products bound to the ribosomes supports this conclusion and demonstrates that 60 per cent of the AcPhe-tRNA and 70 per cent of the Phe-tRNA from complex II are present as AcPhe-Phe-tRNA. The remainder of the products are AcPhe-tRNA and Phe-tRNA; there is no detectable tripeptide.

Table 1. IDENTIFICATION OF PRODUCTS FORMED AS A RESULT OF THE BINDING OF ^{3}H-Phe-tRNA FROM COMPLEX II TO RIBOSOMES CARRYING Ac-^{14}C-Phe-tRNA

Additions to ribosomes carrying Ac-^{14}C-Phe-tRNA	Bound to ribosomes		Released by puromycin		Recovered after alkaline hydrolysis	
	Ac-^{14}C-Phe	^{3}H-Phe	Ac-^{14}C-Phe	^{3}H-Phe	Ac-^{14}C-Phe	Ac-^{14}C-Phe-^{3}H-Phe
(*A*) None	10·7	—	10·2	—	10·8	—
(*B*) ^{3}H-Phe-tRNA in complex II	10·4	8·4	3·0	1·0	3·2	6·0

Results are in pmoles/0·1 mg ribosomes.

Deoxycholate washed ribosomes, transfer factors TI$_s$, TI$_u$, and TII, ^{3}H- and ^{14}C-Phe-tRNA were prepared as previously described[9,17] and stored at −90° C. Initiation factors f$_1$ and f$_2$[18], complex II[9] containing ^{3}H-Phe-tRNA and γ-^{32}P-GTP and Ac-^{14}C-Phe-tRNA[19] were prepared as previously described. (*A*) To prepare ribosomes carrying Ac-^{14}C-Phe-tRNA, 5 mg ribosomes and 0·5 mg poly U were incubated for 5 min at 37° in 1 ml. of buffer 5 (Tris-HCl, *p*H 7·7, 0·05 M; dithiothreitol, 5 mM; NH$_4$Cl, 80 mM; KCl, 80 mM; and MgCl$_2$, 5 mM). The mixture was cooled and f$_1$ (150 μg of protein), f$_2$ (225 μg of protein), GTP (0·5 μmole) and Ac-^{14}C-Phe-tRNA (1·5 nmoles) were added in a final volume of 5 ml. of buffer 5. The mixture was incubated for 15 min at 25°, cooled, and layered over 5 ml. of buffer 5 containing 10 per cent sucrose. The ribosomes were collected by centrifugation at 150,000*g* for 90 min, and the ribosomal pellet was suspended in 2 ml. of buffer 5 and stored at −90° C. (*B*) To 5 mg of ribosomes carrying Ac-^{14}C Phe-tRNA, prepared as described above, was added complex II, containing 1·5 nmoles of ^{3}H-Phe-tRNA and 2·5 nmoles of γ-^{32}P-GTP, and the mixture was incubated for 10 min at 0° C. The ribosomes were collected by centrifugation through buffer 5 containing 10 per cent sucrose and resuspended as described above. The amounts of Ac-^{14}C-Phe-tRNA and ^{3}H-Phe-tRNA bound to ribosomes were measured by the 'Millipore' filter method as previously described[17]. To determine the release by puromycin, 0·2 mg of the ribosomal complexes and 1·0 mM puromycin were incubated for 30 min at 0° C in 0·25 ml. buffer 5, and the amount of ethyl acetate extractable material was determined as described by Leder and Bursztyn[20]. The products bound to 0·8 mg of ribosomal preparations *A* and *B* were liberated by alkaline hydrolysis[10] and identified by chromatography on 'Sephadex *G*-15' equilibrated in 0·5 M acetic acid, a modification of the procedure described by Bretthauer and Golichowski[21].

Table 2. IDENTIFICATION OF PRODUCTS FORMED AS A RESULT OF THE BINDING OF Ac-^{14}C-Phe-tRNA TO RIBOSOMES CARRYING ^{3}H-Phe-tRNA FROM COMPLEX II

Additions to ribosomes carrying ^{3}H-Phe-tRNA	Bound to ribosomes		Released by puromycin		Recovered after alkaline hydrolysis	
	Ac-^{14}C-Phe	^{3}H-Phe	Ac-^{14}C-Phe	^{3}H-Phe	Ac-^{14}C-Phe	Ac-^{14}C-Phe-^{3}H-Phe
(*A*) None	—	6·5	—	0·5	—	—
(*B*) Ac-^{14}C-Phe-tRNA	9·9	5·7	8·8	0·7	9·2	0·3

Results are in pmoles/0·1 mg ribosomes.

(*A*) To prepare ribosomes carrying ^{3}H-Phe-tRNA from complex II, 5 mg of ribosomes and 0·5 mg of poly U were incubated for 5 min at 37° C in 1 ml. of buffer 5. The mixture was cooled and complex II, containing 2 nmoles of ^{3}H-Phe-tRNA and 3·3 nmoles of γ-^{32}P-GTP, was added in a final volume of 5 ml. of buffer 5. The mixture was incubated for 10 min at 0° C and layered over 5 ml. of buffer 5 containing 10 per cent sucrose. The ribosomes were collected and resuspended as described in Table 1. (*B*) To 5 mg of ribosomes carrying ^{3}H-Phe-tRNA from complex II were added f$_1$ (150 μg protein); f$_2$ (225 μg protein); GTP, 0·5 μmole; and Ac-^{14}C-Phe-tRNA, 1·5 nmoles. The ribosomes were collected and determinations were conducted as described in Table 1.

At 5 mM Mg^{2+} Phe-tRNA from complex II is bound to ribosomes with unoccupied donor sites (Table 2), and very little of the bound Phe-tRNA reacts with puromycin. This and other data[9] indicate that the Phe-tRNA is bound at the acceptor sites. The amount of Phe-tRNA bound to ribosomes having empty donor sites rarely exceeds 5–7 pmoles per 0·1 mg ribosomes, whereas 8–10 pmoles are bound to ribosomes carrying AcPhe-tRNA (Table 1). In the conditions described in Tables 1 and 2, approximately one GTP is hydrolysed per Phe-tRNA bound to the ribosome[9,10]. The amount of AcPhe-tRNA bound to the ribosomes in the presence of initiation factors and GTP is approximately 8–10 pmoles whether or not the ribosomes carry Phe-tRNA from complex II. In addition, about the same amount of AcPhe-tRNA is released by puromycin whether or not Phe-tRNA has been previously bound at acceptor sites. When AcPhe-tRNA is bound to ribosomes carrying Phe-tRNA from complex II, less than 10 per cent of the AcPhe-tRNA and the Phe-tRNA interact to form dipeptide (Table 2). Incubation of the ribosomes carrying Phe-tRNA and AcPhe-tRNA with TI$_s$, TI$_u$ and GTP does not decrease the amount of AcPhe-tRNA that reacts with puromycin nor does such a treatment increase the amount of dipeptide. If, however, these ribosomes are incubated with TI$_s$, TI$_u$ and GTP in the presence of exogenous Phe-tRNA, additional Phe-tRNA is bound, and there is a decrease in the amount of AcPhe-tRNA that reacts with puromycin. Analysis of the products bound to the ribosomes indicates that the AcPhe-tRNA has interacted selectively with the exogenous Phe-tRNA to form dipeptide. There is no detectable conversion of the endogenous Phe-tRNA to dipeptide.

Erbe and Leder[22] found that in the presence of the dicodon messenger, AUGU$_3$, Phe-tRNA does not bind to ribosomes at low Mg^{2+} concentrations unless fMet-tRNA is bound to the ribosomes in the presence of initiation factors and GTP. Our results indicate that with poly U, enzymatic binding of Phe-tRNA to acceptor sites can occur at low concentrations of Mg^{2+} when donor sites are unoccupied, but the subsequent addition of AcPhe-tRNA to donor sites does not result in the formation of dipeptide. This observation suggests that binding of AcPhe-tRNA occurs on ribosome–poly U complexes different from those carrying the previously bound Phe-tRNA from complex II. This possibility is supported by the observation that in the presence of TI_s, TI_u and GTP, additional Phe-tRNA is bound to these ribosomes which specifically interacts with the AcPhe-tRNA to form dipeptide. If Phe-tRNA bound at the acceptor site of a ribosome complex prevents the binding of AcPhe-tRNA to the donor site, it is difficult to explain why the presence of the Phe-tRNA does not reduce the total amount of AcPhe-tRNA that is subsequently bound. The existence of populations of different ribosome–poly U complexes is quite likely.

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* In an effort to establish a uniform nomenclature for the transfer factors, the following abbreviations are used:: TI$_s$, TI$_u$, and TII. These correspond to the designations FI$_s$, FI$_u$, and FII[9] and represent enzymatic activities comparable with the factors T$_s$, T$_u$, and G (Lucas-Lenard and Lipmann[11]) and S$_1$, S$_3$, and S$_2$ (Skoultchi *et al.*[7]).

Another possibility that cannot be disregarded is that binding occurs on the same ribosome without dipeptide formation. If this is the case, Phe-tRNA bound at the acceptor site does not interfere appreciably with either the binding of AcPhe-tRNA or the release to puromycin. Binding at the acceptor site without peptide bond formation is also observed when the GTP analogue 5′-guanylmethylene diphosphonate is substituted for GTP[12,13]. If binding occurs on the same ribosome, these data would suggest that the energy of the GTP moiety of the complex II is utilized for bringing the acceptor sites and donor sites into the proper juxtaposition for peptide bond formation and that unless the donor site is occupied at the time of binding (or shortly thereafter) the capacity for the formation of the peptide bond is lost on the release of P_i and $GDP–TI_u$[9,14] from the ribosome.

Whichever possibility is correct, this system furnishes a means of studying the prerequisites for and the mechanism of dipeptide formation. It is tempting to speculate that this system represents a model for a control mechanism that allows only nonfunctional reading of internal codons when the initiator codon has not been read.

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Specific Antibodies to Triiodothyronine Hormone

WE have been able to stimulate the formation in rabbits of a very specific antibody against triiodothyronine (T_3) by injecting the animals with a condensation product of T_3 and succinyl poly-L-lysine. Antibodies against thyroxine (T_4) have been obtained by Clutton, Harington and Yuill[1] and by Churchill and Tapley[2] using conjugated hapten–protein antigens, but the use of natural proteins as carriers may be the reason for the rather low specificity of the resulting antibodies. We prepared succinylated poly-L-lysine by the method of Stason, Vallotton and Haber[3], and reacted it by carbodiimide condensation with the methyl ester of T_3. After saponification a highly specific antigen was obtained.

In a typical procedure, T_3 methyl ester hydrochloride (33 mg), succinylated poly-L-lysine (15 mg), 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (30 mg) and triethylamine (5 mg) were dissolved in dioxane–water (1 : 1; 10 ml.). After being stirred in the dark for 24 h at room temperature, the solvent was evaporated in a vacuum and the methyl ester saponified with 0·1 M NaOH (3·0 ml.); after being washed with water, dioxane and ether–ethanol (75 : 25), 750 μg of the resulting compound in Freund's adjuvant (200 μl.) was injected into the toe-pads of rabbits. A similar subcutaneous injection was given 6 weeks later, and after a further 2 weeks blood was withdrawn from the treated and from normal rabbits. Incubation of dilutions of these sera together with $^{125}I-T_3$ revealed the presence of antibodies directed against T_3.

Table 1. RELATIVE CROSS-REACTIVITY WITH L-TRIIODOTHYRONINE OF THYROID HORMONE ANALOGUES AND OF L-LYSINE

Compound	Relative reactivity (arbitrary value if $L-T_3 = 100$)
$D-T_3$	90–100
$L-T_4$	2–5
$D-T_4$	2–5
3-Monoiodo-L-tyrosine	< 0·1
3,5-Diiodo-L-tyrosine	< 0·1
Deaminothyroxine	< 0·1
Triiodothyroacetic acid	~ 30
L-Lysine	< 0·1

Using the antibody as the saturable reagent, a radioimmunoassay technique for T_3 was developed. Dilutions of T_3 in 0·1 per cent albumin–barbitone were prepared yielding T_3 concentrations of 280, 140, 70, 35, 17·5 and 0 pg per 100 μl. They were placed into incubation tubes together with 20 μl. of $^{125}I-T_3$ (12 pg) and 50 μl. of antiserum (1 : 600 dilution) in albumin–barbitone buffer. After incubation at 4° C for 24 h, 50 μl. of a suspension of charcoal (100 mg per 20 ml.) and methyl-cellulose (100 mg per 20 ml.) in buffer was added. After mixing and standing for 5 min, the mixture was centrifuged for 15 min at 4° C. Radioactivity in both the supernatants (bound fractions) and the charcoal pellets (free fractions) was counted, together with that in the standards, on a two-channel 'Autogamma' counter, and the ratio of the activities was calculated.

Assays of the cross-reactivity of closely related substances are given in Table 1.

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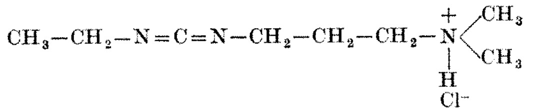
Biologically Active Antibodies to Histamine

It has been known for some time that peptides, amino-acids and proteins containing the substituents COOH and NH_2 can be linked together by the use of water soluble basic carbodiimides[1]. Goodfriend *et al.*[2] showed that linking the polypeptides of bradykinin and angiotensin with rabbit serum albumin (RSA) by the use of carbodiimides produced a complex which was highly antigenic. Subsequently Davis *et al.*[3] showed that the antibodies induced by the bradykinin–RSA complex were protective against the biological effects of bradykinin, and Oken *et al.*[4] obtained similar results with angiotensin. So far the use of carbodiimides has been confined to protein and peptide syntheses.

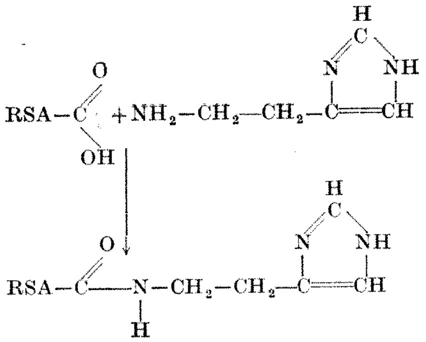
We have made a preliminary study to determine whether or not linkages between RSA and non-peptide compounds containing the NH_2 but not the COOH substituent could be made, and whether or not the resulting complex was antigenic. The compound chosen for the study was histamine, which was linked to RSA using the carbodiimide

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl



$$CH_3{-}CH_2{-}N{=}C{=}N{-}CH_2{-}CH_2{-}CH_2{-}\overset{+}{\underset{\substack{|\\ H\\ Cl^-}}{N}}\begin{matrix}CH_3\\ CH_3\end{matrix}$$

The most likely reaction is between the primary amine of histamine and a carboxyl group from the protein. The chemical reaction is shown as:



The resulting complex was used to immunize guinea-pigs using the immunizing regimen described for bradykinin[2,3] whereby a first immunizing dose is given in the pads of the paws with second and third immunizing doses given intradermally in the back at intervals of 21 days.

Dilutions of the serum which produced a reaction on Ouchterlony plates against the histamine–RSA complex varied between 1 : 16 and 1 : >256, with 80 per cent occurring at dilutions of 1 : 64 or greater. The ability of the antibodies in the serum to neutralize the biological effects of histamine was tested by making a LD_{50} mixture of histamine (0·19 mg/kg body weight for the guinea-pig) either with non-immune serum (control) or with immune serum (test) and injecting these intravenously into guinea-pigs.

Table 1. MODIFICATION OF LD_{50} DOSE OF HISTAMINE BY ANTIBODIES INDUCED BY AN ANTIGEN PRODUCED BY A COMPLEX OF HISTAMINE AND RABBIT SERUM ALBUMIN

			Reaction			
	None	Mild	Moderate	Severe	Death	Total
LD_{50} in non-immune serum						
No. of animals	0	6	4	3	11	24
Percentage	0	25	16	13	46	100
LD_{50} in immune serum						
No. of animals	2	22	6	0	3	33
Percentage	6	67	18	0	9	100

Table 1 shows that the LD_{50} intravenous dose of histamine made up with non-immune serum produced close to the expected 50 per cent death rate with 59 per cent of the animals in the severe reaction and death categories. On the other hand, the LD_{50} intravenous dose of histamine made up with immune serum produced only 9 per cent of animals in the severe reaction and death categories with 73 per cent of animals in the no reaction and mild reaction categories. This shift in distribution was statistically highly significant ($P < 0{\cdot}005$) using the χ^2 test for significance.

The presence of antibodies in actively immunized animals produced no obvious adverse effects in terms of changes in growth rate, overt behaviour or physical appearance. These results indicate that antibodies which neutralize histamine can be induced by an antigen resulting from covalently bonding histamine to a large protein molecule such as RSA.

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Examination of "Species Specificity" of Avian Interferons

INTERFERON preparations usually show some degree of species specificity in their antiviral effects, exerting their greatest effect on growth of viruses in cells of the species which produced the interferon, and being less active or totally inactive in cells of other species[1-3]. Recent studies have shown that species specificity is far from absolute: certain human and primate interferons have proved active in rabbit cells[4]; the activity of monkey interferon in human cells was first noted by Isaacs *et al.*[5]; and cross-reactivity of human and monkey interferon systems at the level of the taxonomic family has been demonstrated[6]. Mouse serum interferon expresses one-twentieth of its mouse cell activity on other rodent cells[7].

These findings suggest that the closeness of the genetic relationship between two species may determine the ability of their interferons to exhibit heterologous activity. To examine this possibility, we have studied the reactivity of avian interferons from three orders of the class *Aves* on cells of closely and distantly related avian species.

Embryo fibroblasts from one-half term embryonated eggs were used, and interferons were produced either in eggs or in the cell cultures. Eggs were inoculated with influenza A–1 virus, strain WS–157. Allantoic fluid was harvested after 48 h incubation. Interferon was produced in cell culture by application of ultraviolet-inactivated Newcastle disease virus at a multiplicity of ten virus plaque forming units (pfu) per cell estimated before irradiation. Culture fluids were prepared after 25 h incubation at 37° C. All preparations were titrated to *p*H 2 with HCl and stored for 48 h at 4° C. After neutralization with NaOH solution, samples were centrifuged at 100,000*g* for 1·5 h. Supernatants were checked for the complete removal of virus by reinoculation into embryonated hens' eggs.

Interferon assays were done on monolayers of embryo fibroblasts in 60 mm Petri dishes. Two-fold dilutions of the samples were applied and left in contact with the cells for 20 h. The cells were than washed with balanced salts solution, and 50 to 80 pfu of vesicular stomatitis virus

Table 1. REACTIVITY OF GALLIFORM INTERFERONS*

	Type of cell culture †								
Order:	Galliformes						Anseriformes		Columbiformes
Family:	Phasianidae				Numididae	Meleagrididae	Anatidae		Columbidae
Type of interferon ‡	Chicken	Pheasant	Chukar partridge	Bob-white quail	Guinea-fowl	Turkey	Pekin duck	Goose	Common pigeon
Chicken (O)	*100*	NSE	80	240	39	13	NSE	NSE	NSE
Turkey (O)	42	37	100	580	82	*86*	NSE	NSE	NSE
Pheasant (O)	22	*120*	140	41	115	58	NSE	—	NSE
Chukar partridge (O)	NSE	NSE	*76*	97	42	19	NSE	—	NSE
Bob-white quail (T)	NSE	NSE	NSE	*92*	NSE	NSE	—	NSE	NSE
Guinea-fowl (O)	NSE	NSE	—	380	*35*	—	—	—	—

* Interferon titres of each preparation on the indicated cell type.
† Classification according to currently accepted scheme[9,10].
‡ Produced in tissue culture (T); produced *in ovo* (O).
NSE = No significant effect; — = Not done.

Table 2. REACTIVITY OF ANSERIFORM AND COLUMBIFORM INTERFERONS*

	Type of cell culture							
	Anseriformes			Galliformes				Columbiformes
Type of interferon †	Pekin duck	Mallard duck	Goose	Chicken	Turkey	Bob-white quail	Guinea-fowl	Common pigeon
Pekin duck (O)	*78*	68	15	NSE	NSE	NSE	NSE	NSE
Mallard duck (O)	70	*73*	13	—	NSE	—	NSE	NSE
Goose (O)	165	36	*30*	NSE	NSE	NSE	NSE	NSE
Common pigeon (T)	NSE	—	NSE	NSE	NSE	—	NSE	*75*

* Interferon titres of each preparation on the indicated cell type.
† Produced in tissue culture (T), produced *in ovo* (O).
NSE = No significant effect; — = Not done.

was applied. After adsorption for 1 h at 37° C, agar overlay was added. When plaques were well developed a second agar overlay containing 0·1 per cent 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) (Aldrich Chemical Company)[8] was added and the plates incubated 8 h or longer—until the living cells reduced the tetrazolium salt to a purple-red formazan and plaques could be counted. Titres of viral inhibitor are expressed as the reciprocal of the dilution of the sample which reduced the plaque count to 50 per cent of that of the controls.

Table 1 shows the results of assaying interferons from various species of the Galliformes. The titres on homologous cells are in italic type. Considerable interspecific activity was found between interferons and cells of the six representatives of the Galliformes. No effect was found when any of the galliform interferons were tested on cells of species from the orders Anseriformes or Columbiformes. Certain interferons, notably quail, displayed a "one way" activity similar to that observed by Desmyter *et al.*[4]. Quail interferon is inactive in all except its homologous cells, but all other galliform interferons are highly active in quail cells.

Table 2 shows the results obtained for interferons from the three anseriform and one columbiform species. Preparations from the Pekin duck, mallard duck, and goose protected homologous cells and cells of the other two anseriform species to some extent, but the other cells not at all.

Tests on several of the heterologously active viral inhibitors have shown that they qualify as interferons by meeting the generally accepted requirements[2,11].

These studies show that degree of genetic relatedness between avian species can influence the ability of their interferons to exhibit heterologous activity. "Order specificity" is indicated: cells and interferon preparations from members of one avian order can interact to some extent with others of their order, in agreement with the conclusions of Bucknall[6] in his study of primate interferons.

It is not possible to predict interactions by phylogenetic relatedness. Chukar partridge interferon was active in turkey cells but inactive in chick. The chukar partridge and chicken are both members of the same family (Phasianidae), but the turkey is classed in a separate family (Meleagrididae). Similarly, the pheasant and chicken are considered more closely related, in currently accepted classification schemes, than any of the other galliform birds studied, yet chick interferon was totally inactive in pheasant cells. Why bob-white quail cells exhibit a notably increased response to chick, turkey and guinea-fowl interferon, but quail interferon is totally inactive in all other galliform cells, is not yet explained. This may be a question involving cell receptors for interferon. Investigations of "one way" reactivities such as this may answer some basic questions on how and whether externally applied interferon enters cells.

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Synthetic Polyanions protect Mice against Intracellular Bacterial Infection

A NUMBER of synthetic polyanions stimulate interferon production[1-7] and these materials are active against infection with viruses and also with parasites of a more complex nature[8-12]. Mice inoculated intraperitoneally with pyran, a random copolymer of divinyl ether and maleic acid anhydride, are resistant to an ordinarily lethal challenge of mengo virus[7]. Such antiviral resistance persists for as long as 60 days in spite of the absence of measurable levels of interferon in the sera of the mice after 4 to 6 days[7,13]. These polyanions modify a number of host defence mechanisms including delayed hypersensitivity[14], antibody response[15] and the number of circulating white blood cells[16]. In view of the suggestion that a common mechanism of host resistance to intracellular pathogens may exist[17], we were interested to determine if the long term resistance against viral challenge observed following administration of such polymers may also be extended to intracellular bacterial infection. In these studies, three anionic polymers*, pyran copolymer, polyacrylic acid and the double stranded synthetic ribonucleic acid poly I·poly C, each conferred resistance to *Listeria monocytogenes* infection in mice.

Pyran conferred resistance to listeria when inoculated by either subcutaneous or intraperitoneal routes. Swiss–Webster female mice, used in all studies, were inoculated with pyran and challenged at varying times thereafter. Mice challenged with listeria intraperitoneally 18 h after receiving pyran by the same route did not manifest resistance and died earlier than control animals. By 4 days, decreased mortality and/or extension of life was noted and this resistance persisted for 2 months, but at a lower level than noted at 1 month (Fig. 1*A*). Mice challenged with listeria intraperitoneally after subcutaneous injection of pyran were also protected (Fig. 1*B*). Mice challenged with listeria subcutaneously showed protection if challenged with listeria at 18 h, 8 days, 1 month and 2 months after receiving pyran intraperitoneally but not at 4 or 6 months (not illustrated). With this combination of inoculation routes, mice which received 50 mg/kg were better protected at 18 h and 8 days than those which received 125 mg/kg, suggesting that the higher doses were associated with a toxicity which is apparent when the bacterial challenge is close to polymer administration. Preliminary experiments indicated, however, that 125 mg/kg gave more protection at later challenge times.

The relationship of the resistance conferred by pyran to its high density of negative charges was tested using another anionic polymer and its amide. Blocking of the negative charges resulted in a loss of the polymer's ability to confer resistance. Whereas mice which had been injected with polyacrylic acid 1 month previously were remarkably resistant to listeria challenge, those inoculated with polyacrylamide died similarly to control mice (Fig. 1*C*).

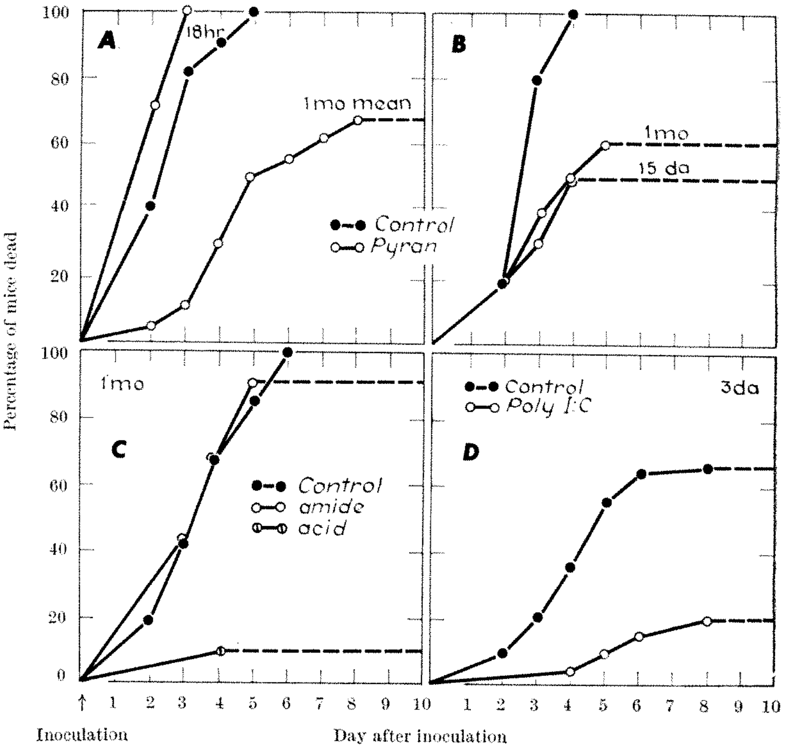

Fig. 1. Effect of anionic polymers on resistance in mice to challenge with *Listeria monocytogenes*. The strain of listeria and preparation of the inoculum were as previously described[17]. Results in *A*, *B* and *C* represent groups of ten mice and in *D* of twenty mice. The time periods represent the interval between injection of the polymer and inoculation of listeria challenge. *A*, 125 mg/kg pyran intraperitoneally and 4×10^7 listeria intraperitoneally; *B*, 125 mg/kg pyran subcutaneously and 4×10^7 listeria intraperitoneally; *C*, 16 mg/kg of polyacrylic acid or of polyacrylamide intraperitoneally and $1{\cdot}5 \times 10^7$ listeria; *D*, 1 mg/kg poly I·poly C intraperitoneally and 9×10^6 listeria intraperitoneally. The results for 1 month in *A* represent the average of results from five separate experiments. Dashed line signifies fraction of mice surviving to 1 month after bacterial challenge.

Poly I·poly C was active in the same experimental model in conferring resistance to listeria, but for a shorter period of time than noted with pyran. Mice were inoculated intraperitoneally with either poly I·poly C or 0·15 M NaCl (the diluent for the poly I·poly C) and challenged with listeria intraperitoneally 18 h, 3 days, 8 days and 14 days thereafter. As observed with pyran, there was early death in mice challenged 18 h after receiving poly I·poly C when compared with controls. At 3 (Fig. 1*D*), 8 and 14 days, protection was apparent in the animals which received the double-stranded polyribonucleotide.

Studies to determine whether resistance might be partially attributable to enhanced reticuloendothelial clearance revealed a significant difference in clearance of carbon between normal mice and pyran treated mice, which were resistant to listeria challenge. The methods used[18] were adapted from the work of Biozzi *et al.*[19]. Carbon particles were injected intravenously into twelve normal and eleven pyran mice (inoculated 30 days earlier with 125 mg/kg pyran). The concentrations of carbon in serial samples of blood were determined spectrophotometrically at 650 nm. Clearance rates (log of the carbon concentration plotted against time) were linear for all animals and the average slope, *K*, was 0·037 (0·026–0·039) for control animals and 0·078 (0·054–0·111) for the pyran treated mice.

Resistance against phylogenetically unrelated organisms has been associated with "activated" macrophages[17]. Because pyran confers resistance to viruses[7], protozoa (in our unpublished studies, when groups of twenty mice were challenged intraperitoneally or subcutaneously with strain C56 of *Toxoplasma gondii* at 18 h and 2 months following intraperitoneal pyran inoculation, decrease in mortality and prolongation of life were noted in groups of pyran treated animals compared with diluent inoculated controls), and bacteria, we investigated whether macrophages from the pyran treated mice were resistant to challenge with listeria *in vitro*. The methods were essentially those previously described[18]. Peritoneal macrophages from mice inoculated intraperitoneally with pyran were resistant to listeria challenge. Whereas control cell monolayers and monolayers from pyran treated mice had comparable numbers of cells per high power field at 1 h after infection, there were more cells infected and more bacteria per cell in the pyran monolayers at that time. At 6 h following infection (in one representative experiment of the eight performed) (Fig. 2), there was a 30 per cent reduction in cells in the normal cell monolayers, but the pyran cell monolayers remained intact. During this time there was an increase in percentage of normal cells infected from 23 per cent at 1 h to 51 per cent at 6 h, but a decrease in the percentage of pyran cells infected from 36 per cent at 1 h to 24 per cent at 6 h. The numbers of stainable bacteria per cell during this time increased in normal cells but remained the same in pyran treated cells.

Similar experiments were performed using macrophages obtained from mice inoculated intraperitoneally 18 h or 3 days earlier with 1 mg/kg of either poly I·poly C, poly I, poly C or diluent. Poly I·poly C and poly I had essentially the same action as pyran in the 18 h mice, but no protection was afforded by poly C. No difference was noted between control cells and those from mice treated with polynucleotides 3 days earlier.

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* Pyran copolymer (mol. wt. 31,000) was obtained through the courtesy of Dr W. H. Wood, of the National Service Center, Drug Development Branch, Cancer Chemotherapy, United States Public Health Service; and polyacrylamide (S-692, mol. wt. 60,000–70,000) and polyacrylic acids (T-132, mol. wt. 60,000–70,000) synthesized by Monsanto, St Louis, Missouri, and obtained from Dr William Regelson, Division of Oncology, Medical College of Virginia, Richmond, Virginia. Poly I·poly C was prepared in our laboratory by annealing homopolyribonucleotides purchased from Miles Laboratories, Elkhart, Indiana.

Identification of the mechanisms of these effects may have useful practical consequences in the control of various intracellular infections in man. The prolonged protective effect of pyran and poly I·poly C in these and other studies[7] suggests mechanisms not involving interferon, because circulating interferon disappears much earlier. The enhanced reticuloendothelial clearance of carbon particles observed in pyran treated animals may not necessarily be critical, for a remarkable resistance of protozoal-infected mice to listeria[17] and mengo virus[20,21] has been observed in the absence of enhanced reticuloendothelial clearance[18]. The "activated" character of macrophages obtained from mice injected with pyran and poly I·poly C suggests a role for these cells in protection against *in vivo* challenge with listeria. To evaluate the contribution of interferon, preliminary experiments were performed using macrophages treated with interferon at levels which protected other cell lines against TRIC agents[10] and *Toxoplasma gondii*[12] as well as viruses. No protection was noted in the interferon treated monolayers. The enhanced antibody production noted following administration of these polymers[15] is not a likely cause of the resistance to listeria infection because most workers[22] agree that conventional circulating antibody does not contribute significantly to host resistance against this intracellular pathogen.

Resistance to intracellular bacteria has been closely related to the presence of delayed hypersensitivity[23]; the resistance conferred by these polymers may be related to their ability to enhance cell mediated defence mechanisms against foreign antigens[14]. The critical antibacterial mechanism(s) operative in this system may be similar to those that mediate the anti-protozoan and anti-tumour effects of these polymers[11,24,25]. Other recent data (ref. 26 and personal communication from M. Weinstein, J. Waitz and P. Came) indicate protective effects with polyanions against other bacterial species in mice.

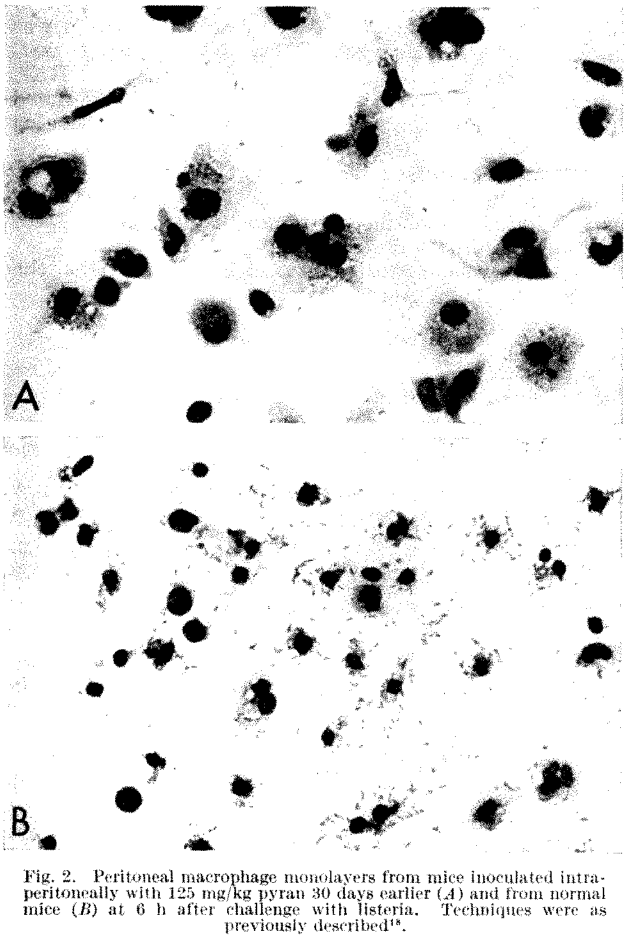

Fig. 2. Peritoneal macrophage monolayers from mice inoculated intraperitoneally with 125 mg/kg pyran 30 days earlier (*A*) and from normal mice (*B*) at 6 h after challenge with listeria. Techniques were as previously described[18].

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Induction of Resistance to Tobacco Mosaic Virus by Poly I·poly C in Plants

PREVIOUSLY it was reported from our laboratory that when yeast RNA was injected intercellularly into leaves of *Nicotiana tabacum* L. Samsun NN, induced resistance developed from the third day on[1]. Interference was highest when plants were inoculated with tobacco mosaic virus (TMV) 5–6 days after RNA was injected at a concentration of 5 mg/ml., and remained high even after 20 days. In the resistant tissue, TMV lesion numbers decreased by 80–90 per cent, compared with those developing in water-injected control tissues. The development of resistance was inhibited if actinomycin *D* was applied shortly after the induction with yeast RNA.

These results were confirmed with yeast RNA and with RNA isolated from plants different from the test species (foreign RNA), whereas the resistance response was not elicited by RNA isolated from plants of the test species[2]. A similarity between yeast RNA induced resistance in plants and interference in animal virology mediated through interferon was suggested[1,2].

Recently, it was reported that double-stranded RNA, polyinosinic-polycytidylic acid (poly I·poly C), is capable of inducing animal cells to produce large amounts of interferon and high levels of resistance to virus infection[3,4]. We were therefore interested to see if poly I·poly C would induce resistance against viruses in plants.

Tobacco NN and *Datura stramonium* L. plants were grown in a screened greenhouse, and transferred before use to a greenhouse chamber set at 20° C, as described previously[1]. Poly I and poly C homopolymers (Schwarz BioResearch or Miles Laboratories) were dissolved in small amounts of sterile double-distilled water and then suspended in 0·15 M NaCl or KCl. Solutions of poly I and poly C were mixed in equimolar concentrations. Complex formation, as determined by hypochromic effect, developed within a few minutes after mixing. The poly I·poly C solution was then diluted to the appropriate concentrations of poly I·poly C and salt, and persistence of the complex was again checked spectrophotometrically. Poly I·poly C, or sterile salt solution as a control, was injected intercellularly into the opposite halves of tobacco NN or *D. stramonium* leaves[1,5]. The leaves were then washed carefully with tap water. For each experiment the two lower leaves of at least three tobacco NN or *D. stramonium* plants were used. In additional control plants the suspending medium was injected into one side of the leaves, whereas the opposite sides remained uninjected. Injections of poly I·poly C (50 μg/ml.) in salt solutions, or salt solutions alone at concentrations between 0·03 and 0·15 M, did not damage the leaves. Occasionally, with the higher salt concentrations, wilting was observed immediately after injection, but the leaves recovered completely within hours. About 0·6–0·9 ml. and 0·3–0·6 ml. fluid was injected into each half leaf of tobacco NN or *D. stramonium* respectively. After varying intervals the whole leaf was inoculated with TMV (5 μg/ml.). Lesions were counted after 7 days and the interference percentage induced by poly I·poly C was calculated. The results were analysed by the "sign test"[6].

Table 1. DEVELOPMENT OF INDUCED INTERFERENCE AFTER INJECTION OF POLY I·POLY C INTO HALF-LEAVES OF TOBACCO NN AND *D. stramonium*

Host	Time between poly I·poly C injection and inoculation with TMV	Lesions* per half-leaf injected with		Interference (per cent)
		Poly I·poly C	Control medium	
Tobacco NN	5–7 h	321	337	4
	24 h	446	697	36 ‡
	2 days	122	238	49 ‡
	3 days	105	169	38 ‡
	4 days	157	213	26 †
D. stramonium	5–7 h	104	113	5
	24 h	224	224	0
	2 days	130	245	47 ‡
	3 days	144	224	36 †
	4 days	226	357	37 ‡
	5 days	64	127	50 ‡
	6 days	145	376	61 ‡

* Average of counts from three experiments, six leaves each.
† Significant at the 5 per cent level.
‡ Significant at the 1 per cent level.

Results, averages from three experiments, are summarized in Table 1. When tobacco NN leaves were inoculated with TMV 5–7 h after poly I·poly C injection, no difference in lesion number between poly I·poly C treated and control half-leaves was observed. If, however, TMV was inoculated 24 and 48 h after poly I·poly C injection, the lesion number on the poly I·poly C injected half-leaves was reduced by 36 and 49 per cent, respectively. In eight additional experiments, when TMV was inoculated 48 h after poly I·poly C injection (in salt concentrations between 0·05–0·15 M), significant reductions (at the 0·01 level) in lesion number were always observed. The variation in the number of lesions in the control half-leaves, especially the increase noted when tobacco NN leaves were inoculated 24 h after injection, does not seem to be significant. It seems to represent a statistical effect caused by variations in sensitivity of plants in different experiments. Occasionally, the reduction in lesion numbers on the poly I·poly C injected leaves reached 70–80 per cent. This was accompanied by a considerable reduction in lesion size. Generally, after 4 days the degree of interference induced in tobacco, by this concentration of poly I·poly C, decreased. In the majority of experiments no significant effects of injecting salt solutions alone, compared with non-injected controls, were observed. Those few experiments in which salt solution affected lesion number by more than 15 per cent were not included in the evaluation of the results obtained by poly I·poly C. Preliminary experiments with lower concentrations of poly I·poly C, that is, 15 and 25 μg/ml., were generally less effective; and concentrations of 100 and 200 μg/ml. did not result in higher levels of interference than those obtained with 50 μg/ml.

Similar results were observed in *D. stramonium* leaves injected with poly I·poly C (50 μg/ml.) in 0·037 M or 0·05 M KCl. Interference was highest, however, when plants were inoculated with TMV 5–6 days after poly I·poly C treatment (Table 1).

Inoculating TMV in the presence of 60 and 200 μg/ml. poly I·poly C did not inhibit lesions, and no interference developed in tobacco leaves if poly I or poly C (50 μg/ml. in 0·075 M NaCl) alone was injected.

These results suggest that the multistranded synthetic polynucleotide complex poly I·poly C activates an interference mechanism in plant hosts, because a time interval is necessary for the development of the resistance, and because no competitive inhibition was observed even at concentrations that were three times higher than those necessary for inducing interference. This induction of interference by poly I·poly C in tobacco NN leaves was less effective than by yeast RNA. Poly I·poly C was, however, active at 50 μg/ml., whereas with yeast RNA 5 mg/ml. was required for induction. It should be noted that for the induction of resistance, the double-stranded polynucleotide was necessary, and no interference developed if single-stranded poly I or poly C at these concentrations was applied. Similar results have been reported for the induction of interferon and resistance to virus infection in animal cells[3,4].

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Increase in Antiviral Activity of Polynucleotides by Thermal Activation

LIMITED preincubation (5 min to 2 h) at 37° C was found to increase the activity of several synthetic polynucleotides in producing cellular resistance to virus infection *in vitro* by several orders of magnitude, and to a lesser extent, induction of interferon *in vitro* and *in vivo*. The thermal activating effect was observed for both alternating copolymers and homopolymer pairs of either RNA or DNA types, but was most marked with the alternating polyribonucleotides such as poly A·poly U and poly I·poly C. Our findings might help to explain the variable biological activity of polynucleotides according to the way they are processed before testing.

Polynucleotides were obtained from different sources: the alternating copolymers poly (I–C), poly (G–C), poly d (I–C) and poly d (G–C) were prepared by one of us (R. D. W.); poly d (A–T) was kindly supplied by Dr I. R. Lehman (Stanford, California). The random copolymer poly (A,U) (1 : 1) was purchased from Miles Laboratories, Elkart, Indiana, and the alternating copolymer poly (A–U) from Biopolymers, Pinebrook, New Jersey. The homopolymer pairs poly I·poly C and poly A·poly U were prepared by annealing the individual homopolymers as described previously[1]. Poly I and poly C were purchased from P–L Biochemicals, Milwaukee, Wisconsin, and poly A and poly U from Miles Laboratories. Concentrations of the polymers were determined spectrophotometrically[1]. They were dissolved in normal saline, stored at –20° C but dialysed at 4° C against minimal Eagle's medium (MEM) before use.

Interferon production and cellular resistance to bovine vesicular stomatitis virus (Indiana strain) were determined in human skin fibroblasts as described previously[1]. Interferon production was also measured *in vivo* (Swiss–Webster mice, 15–20 g) after intravenous injection.

Table 1. CELLULAR RESISTANCE TO VESICULAR STOMATITIS VIRUS IN HUMAN SKIN FIBROBLASTS

	Minimal inhibitory concentration* (μg/ml.)	
Polynucleotide	Preincubation† (2 h) at 0° C	at 37° C
Poly (A–U)	> 1	0·000004
Poly (I–C)	> 10	0·0004
Poly (G–C)	> 10	0·001
Poly A·poly U	0·4	0·01
Poly I·poly C	0·004	0·00004
Poly d (A–T)	⩾ 10	0·01
Poly d (I–C)	⩾ 10	0·04
Poly d (G–C)	⩾ 10	0·1

* Concentration of polymer required to reduce virus plaque formation by 50 per cent.

† 0·15 M Na^+, pH 7·0–7·5 (normal saline, phosphate buffered saline or MEM). Poly (A–U), poly A·poly U and poly I·poly C were preincubated at 1·6 μg/ml., other polymers at 16 μg/ml. All incubations were performed in waterbaths at the indicated temperatures. Samples were further diluted in either precooled (0° C) or prewarmed (37° C) MEM and the dilutions applied to the cell monolayers immediately.

Preincubation at 37° C, compared with 0° C, rendered all polynucleotides, including poly I·poly C, significantly more active in reducing virus plaque formation in human cells, regardless of the medium in which the incubation was carried out (normal saline, phosphate buffered saline or MEM) (Table 1). The antiviral activities of the polydeoxyribonucleotides, which have repeatedly been reported to be low, or non-existent[2-5], were increased after heating at 37° C to values corresponding to those for the unheated poly A·poly U and poly I·poly C. Of all polymers tested, poly (A–U) exhibited the greatest change in antiviral activity. A 5 min incubation at 37° C was sufficient to increase the activity of poly (A–U) by more than 10^6 times. Incubation at 32° C or 56° C was as efficient as incubation at 37° C but there was no activation when poly (A–U) was kept at 25° C. Addition of 10^{-3} M EDTA following the 37° C incubation step or rapid cooling to 0° C (by immersion in solid CO_2–ethanol) reversed the activating effect, whereas slow cooling (by making dilutions in MEM equilibrated at 25° C) did not. Heating poly (A–U) to 90° C (well above its melting temperature[6]), followed by rapid cooling to 0° C, destroyed antiviral activity. Subsequent incubation at 37° C, however, re-established the increased activity. The hypothesis that the activated state of poly (A–U) depends largely on its capacity to form a rigid double helical structure is further supported by findings with the not strictly alternating copolymer poly (A,U). In this copolymer the random distribution of uridine and adenosine does not allow proper base pairing or the formation of a rigid rod-like helical structure, and even after preincubation at 37° C this polymer was devoid of activity at the highest concentration tested (10 μg/ml.).

Preincubation of poly (A–U) and poly (I–C) also increased their interferon inducing capacity *in vitro* (human skin fibroblasts) and *in vivo* (mice) (Table 2), but to a lesser extent than expected from their increased ability to confer cellular resistance to virus infection *in vitro* (Table 1). The homopolymer pair poly A·poly U were not activated and poly I·poly C were only minimally activated when tested for interferon induction either *in vitro* or *in vivo* (Table 2).

Table 2. INTERFERON PRODUCTION

	Interferon units			
	Human skin fibroblasts* (units/4 ml. culture fluid)		Mice† (units/4 ml. serum)	
Polynucleotide	Preincubation‡ (2 h) at 0° C	at 37° C	Preincubation‡ (2 h) at 0° C	at 37° C
Poly (A–U)	4	20	12	24
Poly (I–C)	2	18	15	42
Poly A·poly U	4	3	175	145
Poly I·poly C	10	14	1,310	2,750

* From 3 to 8 h after exposure of confluent cell monolayers in 60 mm Petri dishes to 20 μg of poly (A–U), 30 μg of poly (I–C), 200 μg of poly A·poly U and 20 μg of poly I·poly C in 1 ml. of MEM. Polymers were removed after 3 h incubation; cells exhaustively washed with MEM and further incubated with 4 ml. of MEM per Petri dish.

† 2 h after intravenous injection of 8 μg of poly (A–U), 20 μg of poly (I–C), 100 μg of poly A·poly U and 1 μg of poly I·poly C in 0·5 ml. MEM.

‡ All samples were preincubated in MEM at the given concentrations and applied to the cell monolayers or injected into animals immediately after.

Thus heating to 37° C must promote fundamental alterations in the secondary structure of polynucleotides (particularly alternating polynucleotides) which lead to increased biological activity. Studies to examine the relationship between the increased antiviral activity of preheated polynucleotides and changes in their physical properties and/or sensitivity to exo and endonucleases will be reported elsewhere (our unpublished results).

All polynucleotides tested are capable of forming a double-stranded structure and it is likely that moderate heating increased their antiviral activity by stabilizing their secondary double-stranded structure[1]. This stabilization may have been achieved through movement of one strand relative to its complementary strand[7], which might unite interrupted helices, take up loose, unpaired ends and debranch branched[8,9] structures, thereby reducing the number of unpaired regions.

The final antiviral activity of the thermally activated copolymers poly (A–U) and poly (I–C) was similar to the activity of their thiophosphate analogues, as measured by vesicular stomatitis virus plaque reduction in human skin fibroblasts (ref. 10 and unpublished results of E. de C., F. Eckstein, H. Sternbach and T. C. M.). In terms of interferon production *in vitro* and *in vivo*, however, substitution of thiophosphate for phosphate groups proved much more effective than thermal activation; an average of two to three-fold increase of interferon production was observed for the preheated poly (A–U) and poly (I–C) compared with a twenty to forty-fold increase for their thiophosphate analogues (ref. 10 and unpublished results of E. de C., F. Eckstein, H. Sternbach and T. C. M.). Heating of the thiophosphate analogues of poly (A–U) and poly (I–C) did not cause significant shifts in activity (unpublished results of E. de C., F. Eckstein, H. Sternbach and T. C. M.). Incubation at 37° C and substitution of thiophosphate for phosphate may both lead to enhanced antiviral effect through similar mechanisms or changes in molecular configuration. The heat-induced configurational changes are likely to be more reversible than the changes induced by substituting thiophosphate for phosphate, however, and this may explain the higher activity of the thiophosphates over the heated polymers *in vivo*.

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Anti HL-A2 Inhibitor in Normal Human Serum

THE finding that allogeneic pig liver transplants frequently survive for a significantly prolonged period of time even without immunosuppression has led to a new line of research[1,2]. Calne and his colleagues have shown that the recipient of an allogeneic liver transplant showed partial tolerance towards kidney, and to a lesser extent towards skin transplants, when these were obtained from the same animal as was the liver.

Control experiments indicated that their findings could best be explained by assuming that the liver transplants had induced a state of (partial) specific immunotolerance. Preliminary findings indicate that similar phenomena were observed in the rhesus monkey[3]. If one assumes that the liver transplant induces a state of specific tolerance in the recipient, one could postulate two possible mechanisms for this. First, a tolerance may be established towards the transplant by the lymphocytes. Because this tolerance is not found in the case of kidney and heart transplants, the liver must be capable of inducing a tolerance. Second, the liver may excrete a substance which induces tolerance. Because the tolerance is specific for the individual, the most probable substance responsible could be a soluble HL-A antigen product (or its equivalent in other species)[3]. In that case, one would expect to find HL-A substances in serum or plasma. We describe findings which confirm this hypothesis.

Normal sera, either fresh or stored at 4° C or $-20°$ C, were used. In a few experiments oxalate plasma free of platelets, or sera and plasma, fractionated with either starch block electrophoresis or gel filtration were used. A strong monospecific anti HL-A2 serum (3990) was used throughout. The serum had a titre of about 1 in 32. Specified volumes of sera or plasma, or of fractions of these obtained by 'Sephadex' gel filtration or starch block electrophoresis, were mixed with a volume of the anti HL-A2 serum, incubated in a 37° C water bath for 60 min and thereafter at 4° C for another 60 min. One ml. of this mixture was then tested against 1 µl. HL-A2 positive cells in the microcytotoxicity test described by Kissmeyer-Nielsen[4].

When 0·01 ml. of anti HL-A2 was mixed with 0·20 ml., 0·25 ml. and 0·30 ml. of fresh HL-A2 positive serum and each of these fractions was then tested for residual anti HL-A2 activity, a consistently negative result was obtained only when one part of this particular anti HL-A2 serum was mixed with thirty parts of serum from an HL-A2 positive donor (Table 1). When the donor was HL-A2 negative, clearcut anti HL-A2 activity was always demonstrable, even at this dilution (thirty parts serum from an HL-A2 negative donor mixed with one part anti HL-A2 serum 3990).

Table 1. DETERMINATION OF THE OPTIMAL CONDITIONS FOR THE INHIBITION TEST

HL-A2 type of serum donor	Cytotoxicity with serum volume: 0·20 ml.	0·25 ml.	0·30 ml.
1 positive	+ +	+ +	–
2 positive	+ +	+	–
3 positive	+ +	(+)	–
4 negative	+ +	+ +	+ +
5 negative	+ + +	+ +	+
6 negative	+ + +	+ + +	+ + +

Different amounts of serum to be studied for HL-A2 inhibitory activity were mixed with 0·01 ml. of anti HL-A2 serum 3990, incubated first in a 37° C waterbath for 60 min and then at 4° C for another 60 min. One µl. of this mixture was tested against HL-A2 positive lymphocytes in the microcytotoxicity test[4]. When 0·30 ml. of serum was used for the inhibition test a distinction between sera from HL-A2 positive and negative donors was possible.

Table 2. HL-A2 ACTIVITY IN NORMAL HUMAN SERUM

Sera from HL-A2 positive donors: Donor No.	Storage (days)	Cytotoxicity results	Sera from HL-A2 negative donors: Donor No.	Storage (days)	Cytotoxicity results
1	0	–	25	0	+ +
2	0	–	26	0	+ +
3	0	–	27	0	+ +
4	0	–	28	0	+ +
5	0	–	29	0	+ + +
6	0	–			
7	0	–			
8	0	–			
9	0	–			
10	0	–			
11	0	–			
12	0	–			
13	0	–			
14	0	–			
15	7	–	30	7	+ +
16	7	–	31	7	+ +
17	7	–			
18	30	–	32	30	+ +
19	30	–	33	30	+
20	30	+	34	30	+ + +
21	30	+			
22	30	+			
23	30	+			
24	30	+			

0·30 ml. serum to be tested was mixed with 0·01 ml. anti HL-A2 3990, incubated first at 37° C for 60 min and then at 4° C for another hour. Then 1 µl. of the mixture was tested against 1 µl. of HL-A2 positive lymphocytes in the microcytotoxicity test. A negative reaction implies that inhibitory activity was present; a positive reaction, that it was not present.

A larger number of sera from HL-A2 positive and negative donors was studied (Table 2). It was observed that, in the conditions of the test, sera from HL-A2 negative donors were not able to inhibit the anti HL-A2 activity of the antiserum; sera from most HL-A2 positive donors did so, however. Although in some cases the inhibitory activity was present after prolonged storage at $-20°$ C, the results with fresh serum seemed to be the most consistent.

Similar findings were obtained with oxalate plasma free of platelets, which makes it most unlikely that the inhibitory activity is the result of antigen or hapten release *in vitro* caused by platelet disintegration during clotting. Sera from HL-A2 negative and positive donors are clearly distinguishable only over a narrow range of serum dilutions (Table 1). These dilutions are volumetric. It is thus unlikely—also considering the consistency of our findings—that our observations are the result of a serological artefact.

Residual anti HL-A2 activity after the inhibition test was determined in the microcytotoxicity test described

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PRINCIPLES OF HISTOCHEMISTRY—W. G. Bruce Casselman
DEVELOPMENTAL GENETICS AND ANIMAL PATTERNS—K. C. Sondhi
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by Kissmeyer-Nielsen; human serum was used as a source of complement. Our studies indicate the importance of using human sera from donors who do not carry the antigen under study as a source of complement.

The results obtained by starch block electrophoresis were sometimes erratic. Although a number of sera from HL-A2 positive donors showed consistent and reproducible inhibitory activity of their albumin fractions, others did not.

These preliminary findings make it highly probable that HL-A2 haptens or antigens are present in the plasma and serum of man. Although it is tempting to speculate about a possible correlation between Calne's experiments and our results, it should be stressed that nothing is known at present about the possible biological implications of our findings, if any. Whether similar substances are present in pig serum is also unknown. The study of other antigens has been hindered by the lack of high-titre monospecific antisera. The possible biological and clinical implications of these findings, especially for the induction of specific tolerance, are now the subject of further study.

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Xg Locus: Failure to detect Inactivation in Females with Chronic Myelocytic Leukaemia

THE inactive-*X* hypothesis states that only one of the two *X* chromosomes in each somatic cell of adult mammalian females is genetically active. The initial event in early embryogenesis which determines whether the paternal or the maternal *X* chromosome will remain active in any given cell is presumably random; once made, the choice is fixed for that cell and for all its descendants[1-4]. Although this type of inactivation has been demonstrated in man for a number of *X*-linked loci[5], it is not known whether the entire *X* chromosome is inactivated.

The evidence is particularly conflicting for the *X*-linked locus determining the red cell antigen, Xg^a. The inactive-*X* hypothesis predicts that the red cells in an Xg^a/Xg heterozygote should consist of a mixture of Xg(a+) and Xg(a−) cells. In normal females, however, it has not been possible to demonstrate a mixture of this kind in association with the Xg^a/Xg genotype[6,7]. On the other hand, such mosaicism was reported in a family with *X*-linked anaemia, suggesting that the Xg locus does undergo inactivation[8]. Thus the question remains unresolved.

An alternative approach to this problem involves the study of a clone of red cells derived from a single cell. If random fixed inactivation does occur at the Xg locus, one would expect that in Xg^a/Xg heterozygotes, the red cells of which were derived from a single precursor cell, the phenotype would be either Xg(a+) or Xg(a−) depending on which *X* chromosome was active in that single precursor cell. Thus in contrast to normal heterozygotes, all of whom have the Xg(a+) phenotype, only half of the heterozygotes with clonally derived red cells would be Xg(a+).

In this study, patients with chronic myelocytic leukaemia (CML) were used as the source of clonal red cells. Cytogenetic and genetic evidence support a clonal origin of these red cells. For example, patients with CML usually have a partial deletion of a *G*-group chromosome in their myelogenous cells[9]. This so-called Philadelphia[1] (Ph[1]) chromosome is found in a very high proportion of both granulocyte and erythrocyte precursor cells, suggesting a single cell origin[10-13]. This assumption receives strong support from genetic studies. Thus, normal females heterozygous at the *X*-linked glucose-6-phosphate dehydrogenase (G-6-PD) locus have two red cell populations, each expressing the enzyme controlled by a single G-6-PD allele[14-16]; but in heterozygous CML patients whose fibroblasts have both G-6-PD enzymes, the red cells have only a single type, representing one or the other of their two G-6-PD alleles[17]. Moreover, in the red cells of normal subjects heterozygous at the autosomal 6-phosphogluconate dehydrogenase locus, both alleles are expressed; however, in one CML patient heterozygous at this locus, only one of the two alleles was expressed in the red cells, while both were expressed in the fibroblasts[18]. The most likely explanation for this aberration lies in a genetic mechanism such as deletion in a single precursor cell.

Table 1. Xg^a PHENOTYPES IN THE FAMILIES OF ELEVEN FEMALES WITH CML ASSUMED TO BE Xg^a/Xg HETEROZYGOTES ON GENETIC EVIDENCE

				Xg^a Phenotypes			
				Sons		Daughters	
Subject	Ph[1] chromosome	Patient	Spouse	No. Xg(a+)	No. Xg(a−)	No. Xg(a+)	No. Xg(a−)
C	Present	+	+	3	1	3	0
G	NT	+	NT	2	1	0	0
I	Present	+	+	0	1	1	0
M	Present	+	NT	2	1	1	1
A	Present	+	+	2	1	0	0
V	Present	+	NT	0	2	2	2
GP	NT	+	−	2	2	1	3
NN	Present	+	−	0	2	1	1
OC	NT	+	+	1	1	4	0
FG	Present	+	−	1	1	0	3
HZ	Present	+	+	0	1	1	0

NT, Not tested.

In testing the blood of CML patients for this purpose, the assumption is made that the red cells in peripheral blood reflect the phenotype of the CML clone and not the small number of "contaminating" normal cells which may be present. The validity of this assumption was shown by making an artificial mixture consisting of varying percentages of Xg(a+) and Xg(a−) red cells from normal male hemizygotes and testing them with the same anti-Xg^a and antiglobulin sera used for testing CML patients. It was found that when the proportion of Xg(a−) to Xg(a+) cells was 5 : 1 or more, no agglutination was visible. Thus, even if the Xg(a+) normal cells "contaminating" a clone of Xg(a−) cells made up as much as 16 per cent of the total, the phenotype would still be read as Xg(a−).

Thirty-seven females with typical CML were studied. Thirty-two were found in Mexico and the remainder in Seattle and Baltimore. No patient had been transfused within two months of testing. The Ph[1] chromosome was demonstrated in the myelogenous cells of twenty-six patients, including the two who were children. Karyotypes could not be undertaken in the remaining cases; however, they had the typical findings and course of CML which is usually accompanied by the Ph[1] chromosome. In thirty families, blood specimens from 107 first degree relatives were also tested. Of these thirty patients, twenty had had cytogenetic studies.

Only two subjects with CML were typed as Xg(a−) (both had the Ph[1] chromosome); family studies in both

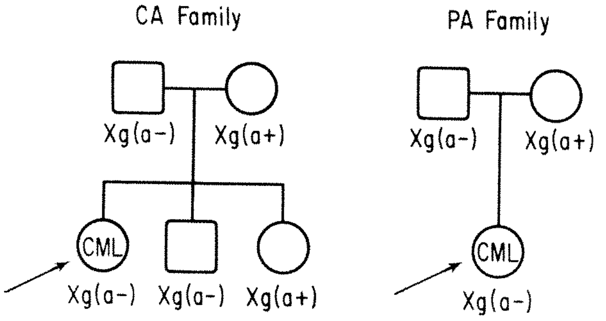

Fig. 1. Pedigrees of two *Xg*(a−) subjects with chronic myelocytic leukaemia.

patients were incompatible with their being *Xg*/*Xg* homozygotes (see Fig. 1). Of the thirty-five females who were typed as Xg(a+), eleven (eight of whom were tested for and found to have the Ph^1 chromosome) were thought to be Xg^a/Xg heterozygotes on the basis of family studies (see Table 1). The results of other genetic marker tests provided no evidence for non-maternity. If random fixed Xg locus inactivation occurs, the probability that all eleven heterozygotes would be Xg(a+) is one in 2,048 (one in 256 if only the eight patients in whom the Ph^1 chromosome was demonstrated are considered).

These findings suggest that the Xg locus does not undergo fixed inactivation in CML red cell stem cells and perhaps not in normal erythrocyte precursor cells either. Nevertheless, alternative possibilities must be considered, such as phenotype alteration due to CML or its therapy, large-scale "contamination" of the CML clone with normal cells, failure of inactivation in abnormal stem cells, non-random inactivation of the *Xg* allele in such stem cells or synthesis of Xg^a antigen in cells other than red cells.

It is unlikely that CML or its therapy changes the Xg phenotype from Xg(a−) to Xg(a+), for two of the Ph^1-positive patients being treated with busulphan were typed as Xg(a−), three of thirteen males with CML were typed as Xg(a−), and no discrepancies in Xg inheritance were encountered in the thirty-nine families studied (thirty families of female and nine of male patients). The possibility of large-scale contamination of the CML clone with normal cells is refuted by the observation that in almost all cases of Ph^1 positive CML more than 85 per cent of the dividing myelogenous cells have the abnormal chromosome[19]. There is no reason to suspect that the initial event leading to CML preferentially occurs in an Xg(a+) stem cell, or that the chromosome carrying the *Xg* gene is non-randomly inactivated. These possibilities, however, cannot be formally excluded. It is conceivable that inactivation at the Xg locus does occur in normal stem cells, but not in the CML progenitor stem cell, or that the locus is reactivated secondary to CML. This would of necessity occur preferentially for the Xg locus, for fixed inactivation at the X-linked G-6-PD locus has been demonstrated in CML cells[17].

From these data we conclude that either the Xg locus does not undergo random fixed inactivation in CML red cells and perhaps in red cells in general, or that the Xg^a antigen is not synthesized in the red cells themselves. Although the latter possibility could explain our observations and those of Gorman *et al.*[6], there is currently no evidence to support a separate site of Xg^a antigen synthesis[7].

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Facilitation of Metastatic Growth by Antilymphocyte Serum

IN 1967, we described the phenomenon of concomitant immunity evoked by a homotransplantable lymphoma in hamsters[1]. Although growing progressively at the site of transplantation, this tumour initiates a strong and specific immune response such that a second (similar) tumour graft is rejected[2]; the immune response is cell-mediated and is reminiscent of a homograft reaction[3]. A second important manifestation of concomitant immunity in tumour-bearing hamsters is the apparent suppression of metastatic growth[1]: viable tumour cells are demonstrable in the blood and regional lymph nodes[4], but there are intense reactive changes in the lymphoid tissues[3,5,6] and metastatic deposits are not established. It follows that if concomitant immunity were weakened in some way, foci of metastatic growth would be expected to appear. One way is to remove the main immunogenic stimulus by excising the tumour 7 days after grafting[7], a procedure which is followed by the development of metastatic deposits in most animals. We now report results indicating that metastatic growth is also favoured by immunosuppression with antilymphocyte serum (ALS).

First, twenty-four young male golden hamsters (75–90 g) from an inbred line (MHA)[8] were used. Cell suspensions of the homotransplantable lymphoma were prepared[3] and 0·5 ml. of a suspension containing 1×10^7 cells/ml. was injected subcutaneously into the right flank of all animals. They were then divided into three equal groups: eight hamsters were treated with a rabbit anti-hamster thymocyte serum prepared according to the method of Levey and Medawar[9]; eight were treated with normal rabbit serum (NRS); and eight received culture medium 'M 199'. Injections were given intraperitoneally, undiluted, in doses of 0·25 ml. on days 1, 3, 6, 10 and 13 after inoculation of tumour. On day 20, peritoneal exudates were raised in all animals by injecting 3 per cent starch solution[10]; the peritoneal cells were collected on day 22, and the hamsters were then killed. Autopsies revealed, first, that the tumour grafts removed from ALS-treated animals were larger than those from control hamsters (injected either with normal rabbit serum or 'M 199') and showed less variation in weight (Fig. 1). This suggests that tumour growth is more uniform when the immune response is impaired. Second, metastic deposits were found in five out of eight hamsters treated with ALS but in none of the control animals. They occurred in the axillary, mediastinal and perirenal lymph nodes and in the periscapular brown fat; no metastases were seen in the lungs or liver.

Concomitant immunity was tested on peritoneal exudate cells in cell transfer tests[11]. In addition to the three groups of eight tumour-bearing animals, six normal MHA hamsters without tumours were also injected intraperitoneally with 3 per cent starch. Suspensions of peritoneal exudate cells from these thirty animals were prepared, mixed with lymphoma cells in a proportion of 2 : 1, and 6×10^6 cells were injected subcutaneously into eighteen normal isogenic MHA recipients. Peritoneal exudate cells from tumour-bearing hamsters treated with ALS were ineffective in suppressing tumour growth in the recipients (Table 1). By contrast, immunity in tumour-bearing animals injected with normal rabbit serum remained intact and their exudate cells completely suppressed tumour growth in the recipients. Treatment with ALS seems to weaken tumour immunity but does not abolish it.

Table 1. DEPRESSION OF TUMOUR IMMUNITY AFTER TREATMENT WITH ALS DEMONSTRATED BY CELL TRANSFER TESTS

Test source of peritoneal exudate cells	No. of recipients developing tumours
Tumour-bearing hamsters treated with ALS	4/6
Controls	
Tumour-bearing hamsters treated with NRS	0/6
Normal hamsters	6/6

Because the tumour under study is a lymphoma, we investigated whether ALS acts mainly on the tumour or on the immune cells. Peritoneal exudate cells were obtained from eight hamsters, inoculated 14 days previously with 5×10^6 tumour cells, and from six normal hamsters. The cells were incubated for 1 h at room temperature in 1 ml. ALS, 1 ml. NRS or in 1 ml. 'M 199'. The tumour cells were similarly incubated in 1 ml. ALS or 1 ml. 'M 199'. ALS and NRS were always heat-inactivated and diluted 1 : 10 before use. There was no evidence of cytotoxicity according to the trypan blue exclusion test[3]. The cells were then washed three times and mixed (see Table 2). As in the previous experiment, a ratio of exudate cells to lymphoma cells of 2 : 1 was fixed and a total of 6×10^6 cells was injected subcutaneously into normal MHA recipients. The results (Table 2) indicate that ALS does not act directly on the lymphoma cells but on the peritoneal exudate cells whose normal ability to suppress tumour growth in isogenic recipients is impaired after treatment *in vitro* with ALS.

The present results illustrate another way in which ALS may potentiate malignant disease. The action of oncogenic viruses such as SV40, Rauscher leukaemia virus and polyoma is potentiated in mice treated with ALS[12–14]. There is some evidence that ALS may augment the effect of chemical carcinogens[15,16] and it may also favour the growth of allogeneic tumour transplants[17,18]. Our results, together with those of Fisher, Soliman and Fisher[19], make it clear that, in certain circumstances, ALS can facilitate metastatic growth. This seems to be a consequence of immunosuppression rather than any direct action on the lymphoma cells themselves, but the identity of the relevant target cells is uncertain. The noncommittal term "peritoneal exudate cells" has been used deliberately because such exudates always contain 5–10 per cent of small lymphocytes. It has not been established whether ALS acts only against this minority of lymphocytes, or against both lymphocytes and macrophages. Some reports[20,21] suggest that ALS may indeed have anti-macrophage activity, but the evidence is conflicting[22].

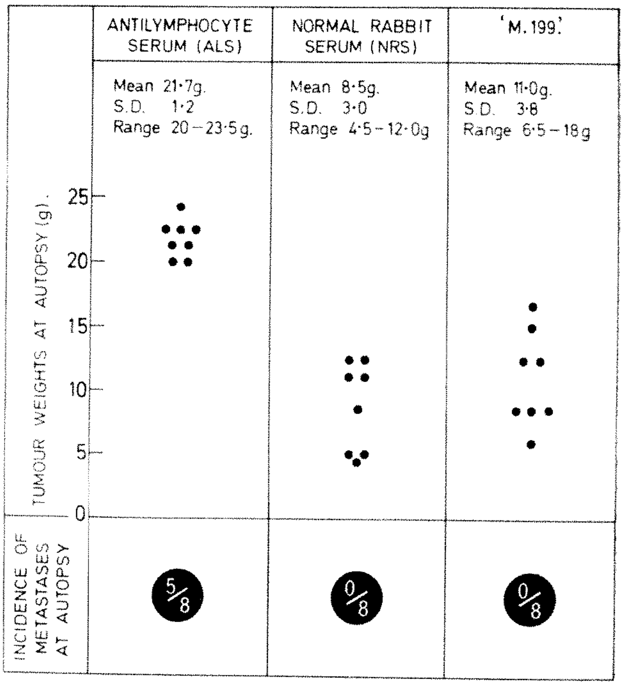

Fig. 1. Tumour weights and incidence of metastases in hamsters treated with ALS, NRS or 'M 199'; 22 days.

Extrapolation of the present results to man would be premature, but there is growing evidence that patients maintained on long-term immunosuppressive treatment may be prone to develop neoplasia[23–25]. Most of the tumours so far reported have been lymphomata; if these lesions show any unusual tendency to disseminate, a relationship in man between prolonged immunosuppression and enhanced metastatic growth may well emerge.

Table 2. CELL TRANSFER TESTS DESIGNED TO DEMONSTRATE SITE OF ACTION OF ALS

Source of peritoneal exudate cells	Tumour cells	No. of recipients developing tumours
(*a*) From tumour-bearing animals		
Treated with ALS	Untreated ('M 199')	5/6*
Treated with NRS	Untreated ('M 199')	0/6
Untreated ('M 199')	Treated with ALS	0/6
(*b*) From normal animals		
Untreated ('M 199')	Untreated ('M 199')	12/12

* Tumours measuring 0·7–1·0 cm in diameter developed at 1 week after inoculation, but they all regressed 1 week later. At this time the immune status of all the recipients listed in this table was tested by transfer tests with peritoneal exudate cells. With a ratio of three exudate cells to one tumour cell, it was found that cells from animals which had previously developed tumours completely inhibited tumour growth in the new recipients ; cells from hamsters which had not developed tumours were without effect. This suggests that the five recipients shown in the first entry on the table were indeed immunized and that their state of immunity was probably responsible for the subsequent tumour regression which was observed. The results also suggest that the inactivation of immune cells brought about by ALS was not, in the present circumstances, complete.

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Desensitization to Acetylcholine of Frog Tonic Muscle Fibres

IT is known that depolarization of the postsynaptic membrane of muscle fibres caused by acetylcholine gradually decreases as a result of its prolonged action: after some time the membrane potential may attain its initial level despite the presence of acetylcholine. This decrease in the sensitivity of the postsynaptic membrane —desensitization—has been investigated either in phasic (fast) muscle fibres of the frog or in mammalian muscle fibres[1-3]. Neither type of muscle fibre develops permanent contraction in response to the action of acetylcholine. We report here findings relating to desensitization in tonic (slow) muscle fibres of the frog which are known to be responsible for acetylcholine induced contraction of the whole muscle.

The experiments were performed on single tonic fibres isolated from the ileofibularis muscle of the frog, *Rana temporaria*, during January and February. The tonic fibres were distinguished by their ability to maintain tension at high external potassium concentrations (for details see ref. 4). The isolated fibre was fixed in a chamber specially arranged for microelectrode investigations. Ringer solution (in mM): Na^+, 111; K^+, 2·5; Ca^{2+}, 1·8; Cl^-, 120·6; HCO_3^-, 2·4. Desensitization was evoked and registered by the method of Katz and Thesleff[2]. Both channels of the double-barrelled micropipette were filled with 2 M acetylcholine chloride.

In all the experiments on eleven end-plates (nine isolated tonic fibres) the amplitude of test responses to brief pulses of acetylcholine from one channel of the double-barrelled micropipette slowly decreased when a light conditioning dose of acetylcholine from the other was given (Fig. 1). The rate of decrease was found to depend on the amplitude of the conditioning depolarization: the greater the amplitude, the faster the decrease of test responses. The rate of reduction of the responses induced by various amplitudes of conditioning dose is similar to that described for fibres from the sartorius muscle of the frog[2,5-7]. The rate of recovery of the test response amplitude after switching off the conditioning current was relatively constant and independent of the onset rate of desensitization (Fig. 1), which is also in accordance with the results obtained for the phasic fibres[2].

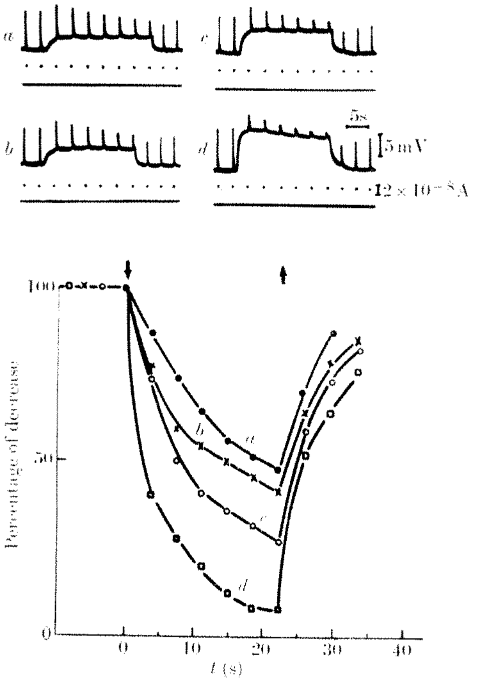

Fig. 1. Desensitization produced by different conditioning doses of acetylcholine at a single end-plate spot of tonic fibre. *Top:* intracellular records of acetylcholine potentials (upper trace). Test pulses and conditioning currents of different strengths were passed through separate channels of a double-barrelled micropipette containing acetylcholine. Lower trace: monitoring of the current through the micropipette containing acetylcholine. *Bottom:* graph showing the rate of onset and recovery of acetylcholine desensitization. The curves were plotted according to corresponding records (*a–d*). Ordinate: relative amplitude of test response; abscissa: time (s). The arrows show the commencement and end of the conditioning current.

To determine the role of desensitization in the mechanism whereby tension is maintained by tonic fibres in the presence of acetylcholine, a simultaneous registration of changes in membrane potential and mechanical tension of these fibres was carried out. Isometric tension was recorded using the mechano-electric transducer 'RCA 5734'. Substitution of the Ringer solution for one containing 2×10^{-5} M acetylcholine caused a decrease in potential of 27–45 mV and a decrease in the tension developed by the fibres. The depolarization amplitude and tension began to fall rapidly even though acetylcholine remained present in the bath (Fig. 2). The same result was obtained with carbocholine (1×10^{-4} M), a cholinomimetic compound not hydrolysed by cholinesterase: a gradual decrease in the effect of acetylcholine is evidently not related to its destruction by cholinesterase.

In control experiments on the same fibres the degree of polarization and tension caused by the elevation of $[K^+]_0$ to 80 mM does not diminish within 1 h. Thus the decrease in tonic fibre contraction induced by acetylcholine is not caused by peculiarities of contraction nor by membrane-myofibrillar coupling mechanisms.

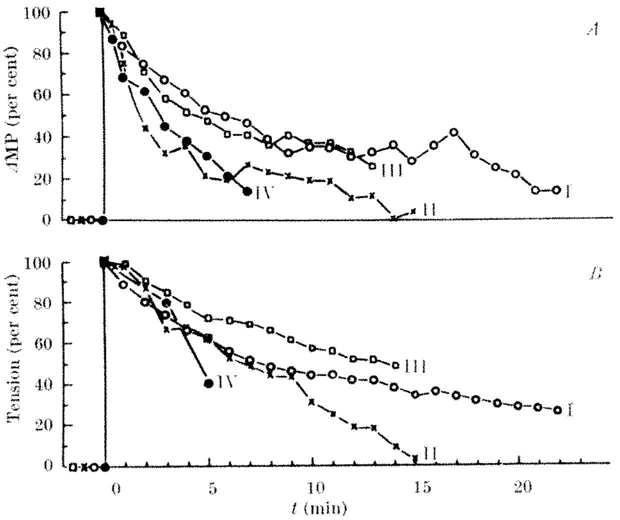

Fig. 2. Simultaneous changes in (*A*) membrane potential (ΔMP) and (*B*) contraction of single tonic fibres in a bathing solution of acetylcholine (2×10^{-5} M). I–IV: individual fibres. The zero on the time axis denotes the onset of the action of acetylcholine.

One cannot overlook the fact that it takes the same time for the membrane potential of tonic fibres and of phasic fibres to return to the initial level in the presence of acetylcholine. Frog tonic fibres are sensitive to acetylcholine only in certain limited zones of their surface corresponding with the points of innervation of these fibres[8]. The areas of these zones are less for tonic than for phasic fibres. Because the rate of desensitization in the former is not less than that in the latter, the ability of tonic fibres to contract under the influence of acetylcholine cannot be assigned to any specific characteristic of their cholinoreceptive zones. These fibres have some synapses located at a distance less than the length constant of their surface membrane[8,9], so that the whole membrane seems to be depolarized electrotonically under the action of acetylcholine. The peculiar character of membrane-myofibrillar coupling helps to maintain contraction for a long period of time[10]. Even so, it lasts only as long as the depolarization of the fibre membrane is maintained: that is, in the case of the acetylcholine effect, up to the onset of desensitization.

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Abnormalities in Platelet 5-Hydroxytryptamine Efflux in Patients with Infantile Autism

AMONG the heterogeneous group of children showing autistic symptoms, Kanner described a small sub-group with what he termed "infantile autism"[1]. Rimland[2] suggested that the reticular formation, known to contain 5-hydroxytryptamine (5-HT) (ref. 3), may be involved.

The blood platelet may be used as a partial model for the 5-HT containing neurone[4]: both cells transport 5-HT by an energy dependent process, store it in subcellular organelles of similar electron microscopic appearance and metabolize 5-HT in a similar way[5]. If the platelet model is valid, then the results described here may indicate that 5-HT is involved in a severe psychotic illness.

The subjects were six children aged 4 to 14, diagnosed by Rimland as having infantile autism[6]. No patient had signs of organic brain disease; no drugs were given for one month before experiment. Platelet 5-HT determinations in six autistic and six normal children of comparable age were made in triplicate, as described by two of us[7]; ATP was measured by the technique of Holmsen, Holmsen and Bernhardsen[8]. Platelets were counted with a 'Model B' Coulter counter (Coulter Electronics, Hialeah, Florida), and the cell size determined with the instrument and with thrombocytocrits[9].

In experiments where 5-HT uptake was investigated, platelet rich plasma was incubated with 10^{-5} M ^{14}C–5-HT (specific activity 9·6 mCi/mmole) for 90 min at 37° C in 5 per cent CO_2 : 95 per cent O_2 to load the cells with amine[7]. The degree of 5-HT binding to the platelets was assessed by resuspending the 5-HT loaded cells in fresh plasma from the same subject and reincubating at 37° C for 90 min. At the end of all uptake and efflux experiments, the platelets were separated from plasma by centrifugation, and total radioactivity in platelets and plasma was estimated by liquid scintillation spectrometry. Values refer to the mean ± standard error of the mean.

Table 1. SIZE, NUMBER, 5-HT CONTENT AND UPTAKE CAPACITY OF PLATELETS FROM NORMAL AND AUTISTIC CHILDREN

	Normal	Autistic
Mean platelet size (μm)^3	19·3 ± 1·2	19·8 ± 1·1
Cells/ml. plasma ($\times 10^8$)	3·51 ± 0·22	4·38* ± 0·36
Endogenous 5-HT (nmoles 5-HT/10^{11} platelets)	1,064 ± 94	838 ± 104
Uptake capacity (net uptake of ^{14}C–5-HT; nmoles 5-HT/10^{11} platelets)	1,080 ± 97	1,371 ± 66
ATP μmoles/10^{11} platelets	5·53 ± 0·51	6·65 ± 0·74
ATP : 5-HT	2·58	3·01

* $P < 0·05$.

Values are the mean ± standard error of the mean of six determinations (made in triplicate) with six normal and six autistic children. The ratios ATP : 5-HT are calculated from the values for total 5-HT (endogenous + uptake capacity) given in the table.

The mean platelet size was not changed in autistic subjects (Table 1); but the number of platelets/ml. plasma was significantly increased. There was no concomitant increase in endogenous 5-HT; rather the concentration of amine was slightly diminished. Platelet ATP was also unchanged, and the ATP : 5-HT ratio was normal.

Autistic platelets accumulated 5-HT to a slightly greater extent than normal, 2·6 times the endogenous content, compared with 2·0 times for normal platelets. This was associated with a slight but not significant increase in ATP. More tests would be required to show whether the autistic platelets were saturated, so the ATP : 5-HT ratio must be considered tentative for autistic cells. Our procedure is known to saturate normal platelets with 5-HT (ref. 10).

Although the 5-HT uptake capacity was unchanged or increased in platelets from autistic subjects, their ability to retain the amine was defective. First, normal and autistic cells loaded with ^{14}C–5-HT lost a similar percentage and total amount of radioactivity during the resuspension procedure alone, before reincubation for 90 min (Fig. 1), indicating that the experimental technique did not increase the fragility of autistic platelets more than normal cells. Consequently, the two-fold increase in efflux of radioactivity from autistic platelets (Fig. 1) represents a definite abnormality, but it did not arise from lack of ATP resulting in diminished binding of 5-HT. Because of the limited opportunity to take blood, we do not know whether the efflux of radioactivity represented 5-HT, 5-hydroxyindolylacetic acid or 5-hydroxytryptophol, an important metabolite of platelet 5-HT (ref. 4).

There are few reports of biochemical studies of 5-HT in autistic children and none where an unequivocal diagnosis of primary infantile autism was made[11-14]. The effects described include reduced excretion of 5-hydroxyindolylacetic acid after a tryptophan load[11]; elevated blood 5-HT (ref. 12) and reduced uptake of 5-HT (ref. 14) by platelets.

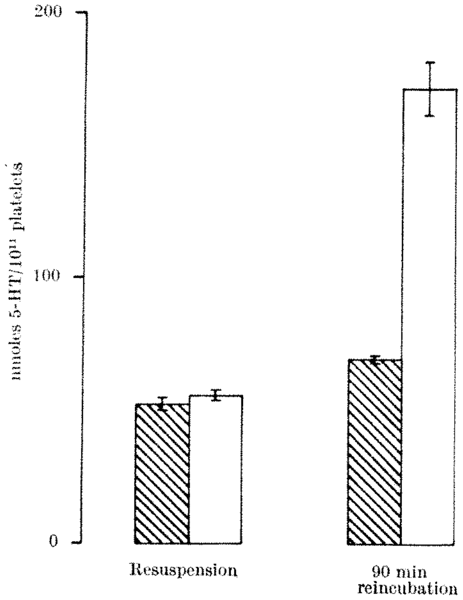

Fig. 1. Efflux of radioactivity. Hatched columns, normal; white columns, autistics.

Because we find a marginal increase in 5-HT uptake and platelet ATP, the increased efflux of 5-HT does not arise from lack of ATP but from some other effect related to 5-HT or its metabolites. In this regard the effects differ from those seen in Down's syndrome where there is also efflux of 5-HT from platelets, which results from defective binding, as there is a lack of ATP (ref. 15).

Thus it must not be assumed that increased efflux of platelet 5-HT is confined to autism; but if the platelet is a valid model for the serotoninergic brain neurone, abnormalities in 5-HT synthesis, binding metabolism and turnover may play a part in autism and other psychotic syndromes.

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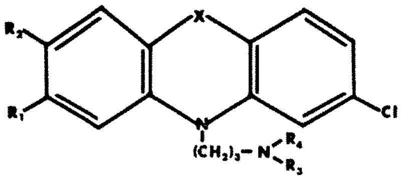
Sensitive Quantitative Determination of Chlorpromazine Metabolites

DIMETHYLAMINONAPHTHYL sulphonyl chloride (dansyl chloride) has been used in recent years as an end group analytical reagent in the study of protein structure[1] and in the determination of amino-acids[2-4]. Dansyl chloride reacts with primary and secondary amines and with phenolic hydroxyl groups[4]. Chlorpromazine, after administration to patients, yields over twenty known metabolites[5]. At least ten of these metabolites possess either an amino or a phenolic hydroxyl group, or both, as potential reactive sites for dansyl chloride coupling. We have studied the reactivity of dansyl chloride with the chlorpromazine metabolites listed in Table 1.

To 0·314 μmoles of the metabolite dissolved in 0·1 ml. of acetone in a 'Pyrex' test tube were added 0·1 ml. of 1·48 μM dansyl chloride in acetone and 10 μl. of 0·3 M sodium phosphate–NaOH buffer (pH 12). The tube was stoppered and shaken overnight. Twenty ml. of the reaction mixture was spotted on each of several 100 μm Eastman thin layer chromatography plates (20 cm × 20 cm) and many different solvent systems were studied to obtain the optimum fractionation. Table 2 includes the solvent mixtures appropriate to the separation of various metabolites with their corresponding R_f values. Reaction products were located by the appearance of brilliantly fluorescent spots on thin layer chromatograms which on spraying with the Forrest reagent[6] became purple, confirming the presence of a phenothiazine nucleus in the

Table 1. VARIOUS CHLORPROMAZINE METABOLITES REACTIVE WITH DANSYL



Metabolite	Abbreviation	R_1	R_2	R_3	R_4	X
7-Hydroxychlorpromazine	7-OH CPZ	H	OH	CH_3	CH_3	S
8-Hydroxychlorpromazine	8-OH CPZ	OH	H	CH_3	CH_3	S
Didesmethylchlorpromazine	Nor_2CPZ	H	H	H	H	S
Monodesmethylchlorpromazine	Nor_1CPZ	H	H	H	CH_3	S
7-Hydroxychlorpromazine sulphoxide	7-OH CPZ-SO	H	OH	CH_3	CH_3	S→O
Didesmethylchlorpromazine sulphoxide	Nor_2CPZ-SO	H	H	H	H	S→O
Monodesmethylchlorpromazine sulphoxide	Nor_1CPZ-SO	H	H	H	CH_3	S→O
7-Hydroxydidesmethylchlorpromazine	7-OH Nor_2CPZ	H	OH	H	H	S
7-Hydroxymonodesmethylchlorpromazine	7-OH Nor_1CPZ	H	OH	H	CH_3	S
7-Hydroxymonodesmethylchlorpromazine sulphoxide	7-OH Nor_1CPZ-SO	H	OH	H	CH_3	S→O

Table 2. SOLVENT SYSTEMS AND R_f VALUES FOR THIN LAYER CHROMATOGRAPHY OF DANSYL PRODUCTS

Benzene–acetone (1 : 1) Product	R_f	Benzene–acetone (9 : 1) Product	R_f	Benzene–acetone (12 : 1) Product	R_f
Dansyl-8-OH CPZ	0·67	Dansyl-Nor$_1$CPZ	0·84	Dansyl-7-OH Nor$_1$CPZ	0·70
Dansyl-7-OH CPZ	0·50	Dansyl-Nor$_1$CPZ-SO	0·37	Dansyl-7-OH Nor$_2$CPZ	0·59
Dansyl-7-OH CPZ-SO	0·39	Dansyl-Nor$_2$CPZ	0·74	Dansyl-7-OH Nor$_1$CPZ-SO	0·36
Dansyl chloride	0·95	Dansyl-Nor$_2$CPZ-SO	0·25	Dansyl chloride	0·92
Dansyl sulphonamide	0·84	Dansyl chloride	0·89	Dansyl sulphonamide	0·26
Dansyl sulphonic acid	0·07	Dansyl sulphonamide	0·46	Dansyl sulphonic acid	0·00
		Dansyl sulphonic acid	0·00		

For explanation of abbreviations of metabolites, see Table 1.

spot. The fluorescent colour of the spot as well as its R_f value were clearly different from those of the reactants and the side product(s)—dansyl sulphonamide and dansyl sulphonic acid.

Dansyl metabolite spots were cut out and extracted with spectrofluorometric grade ethanol (prepared by distilling ethanol over solid KOH) with the help of a 'Vortex' shaker. The excitation and fluorescence maxima of the extracts were determined on an 'Aminco Bowman' spectrophotofluorometer, with stabilized xenon lamp as the light source and an X-Y plotter as the recorder. As little as $3{\cdot}14 \times 10^{-10}$–$3{\cdot}14 \times 10^{-9}$ g of the metabolite was quantitated when different dilutions of the dansyl metabolite products were estimated on a Turner 'Model 110' fluorometer fitted with the proper filters and a high sensitivity door.

Further details will be published elsewhere. We are applying this method of quantitation to study the kinetics of appearance of the chlorpromazine metabolites in the body fluids of animals and schizophrenic patients.

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Inhibition of Mauthner Cells by Allylglycine

IN view of the possibility that glycine or glycine-like substances are inhibitory transmitters in the vertebrate central nervous system (CNS)[1,2], it is particularly interesting that the derivative, allylglycine (2-amino-4-pentenoic acid), causes convulsions when injected into mice and rats[3,4]. Last year it was reported that allylglycine *in vitro* and *in vivo* reduced the synthesis of the depressant amino-acid, γ-amino-butyric acid (GABA)[5], which has itself been suggested as a neural inhibitory substance[6,7]; it seemed possible therefore that the convulsant properties of allylglycine may be due to a reduction in the concentration of GABA which it produces within the brain[5]. This communication describes an investigation of allylglycine on the Mauthner cells of goldfish, and in particular any action it may have either directly on these cells or on inhibitory pathways converging on them.

Goldfish (*Carassius auratus*) weighing 80–120 g were kept at 8°–20° C and injected intramuscularly with 10–15 mg of allylglycine (0·5 M in isotonic saline). Usually within 10–24 h the fish developed a characteristic behaviour consisting of erratic, powerful and apparently uncontrolled swimming, interrupted by relatively quiet periods (lasting about 10 min) during which there were forceful respiratory movements of the jaws and gills. This total activity was judged to correspond to the convulsive behaviour which develops in rats about 2 h after similar injections. The interval between the injections and the development of "convulsions" in the fish was reduced to 2–3 h if the fish were maintained at 30° C. Fish in which convulsions had been induced by allylglycine were anaesthetized with MS222 (Sandoz) and prepared for recording. Glass micropipettes filled with potassium chloride and citrate were used to record intracellularly from the Mauthner cells and in some experiments double and triple barrelled micropipettes filled with glycine (2·5 M, *p*H 3), glutamate (2 M, *p*H 7), and allylglycine (1 M, *p*H 4·5) were positioned close to the cells for extracellular iontophoretic injections. Two inhibitory inputs were investigated, the late collateral inhibition (LCI) and the contralateral VIII nerve inhibition[8], both in the "strychnine-sensitive" category[9,10]. The change in conductance of the cell membrane, measured by the decrease in antidromic spike amplitude, was a convenient measure of inhibition[8-11].

The results indicated quite clearly that the duration and extent of both LCI and VIII nerve inhibitions were

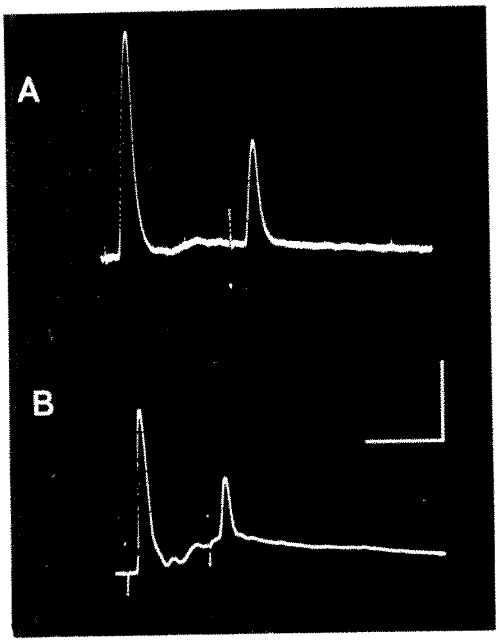

Fig. 1. Physiological inhibition of Mauthner cells in normal fish (*A*) and "convulsant" fish injected with allylglycine (*B*). The traces show the late collateral inhibition (LCI) evoked by a conditioning spike and tested by a second antidromic spike after a delay of a few milliseconds. Inhibitory conductance change is measured by the decreased amplitude of the test spike. The inhibition is similar for both animals. The VIII nerve inhibition (not shown) was also similar for both preparations. The convulsant fish (record *B*) had been injected with 12 mg of allylglycine. Calibrations were 10 mV (vertical) and 4 ms (horizontal).

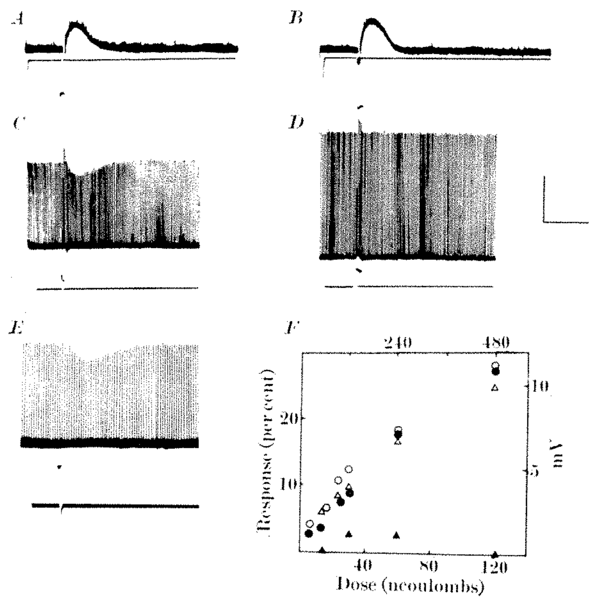

Fig. 2. Effects of iontophoretically applied glutamate, allylglycine, and glycine on the Mauthner cell, before and after injection of strychnine. *A* and *B* show the membrane depolarization (upper trace) caused by a brief pulse of current applied through the glutamate barrel (downwards deflexion in lower trace). *C*, *D* and *E* show records made intracellularly of antidromic spikes (upper trace) and iontophoretic currents applied through the pipette containing the drug (upwards deflexion in lower trace); a decrease in spike amplitude is a measure of the resultant inhibitory conductance change. *C* and *D* are the effects of iontophoretically applied allylglycine, and for comparison, *E* shows the effects of glycine (note different time scale). *A*, *C*, and *E* were taken before, and *B* and *D* after intramuscular injection of 3 mg of strychnine. *F* summarizes the dose response relations of glutamate and allylglycine before and after strychnine had been given; ○, glutamate control; △, allylglycine control; ●, glutamate after strychnine, ▲, allylglycine after strychnine. In *F*, the abscissa is the dose in coulombs $\times 10^{-9}$ (glutamate on top axis, allylglycine on bottom) and the ordinate is the response (mV depolarization for glutamate on the right, percentage reduction of antidromic spike amplitude for allylglycine on the left). Calibrations are: vertical bar, 10 mV and 5×10^{-7} A, and horizontal bar, 4 s for *A*, *B*, *C*, *D* and 2 s for *E*.

normal in convulsant fish (Fig. 1). When iontophoretically ejected on to the cells of normal fish, allylglycine had a direct inhibitory action similar to that of both glycine and GABA, that is, it produced a marked change in conductance with little concomitant change in membrane potential (when recorded with pipettes of potassium citrate). To test whether the actions of allylglycine, like those of glycine itself, are sensitive to strychnine, intramuscular injections of the alkaloid were administered in doses sufficient to block the effects of glycine[10]. Fig. 2 shows, on the left, typical effects of glutamate, allylglycine and glycine before strychnine was injected. Glutamate and allylglycine were applied from a double barrelled iontophoretic pipette near the cell; the typical glycine response from a different Mauthner cell is included to show the similarity of its effects with those of allylglycine. The right side of the figure shows records taken after the injection of 3 mg of strychnine. After strychnine, both glutamate and allylglycine iontophoretic ejection currents were slightly greater, and the spike amplitude increased. The effects of allyglycine were clearly blocked by strychnine. Fig. 2 shows the entire dose-response relations of iontophoretically applied glutamate and allylglycine from a double barrelled pipette before and after injection of strychnine. The concentration of strychnine in the brain was presumably increasing while the records in Fig. 2*F* were being made, which accounted for the complete block of the last response to allylglycine (the largest dose shown in the graph). The lack of any significant alteration in the two glutamate dose-response curves indicates that the results cannot be explained by the movement of the double barrelled pipette containing the drug away from the cell.

It seems therefore that allylglycine can produce "convulsions" in goldfish, but during the convulsive period the collateral and VIII nerve inhibitions are normal. These experiments do not support the interpretation that the convulsions are caused by alterations in inhibitory processes which are sensitive to strychnine. Furthermore, iontophoretically applied allylglycine has a direct inhibitory effect on the membrane which is blocked by strychnine. In view of this, and of the similarity in structure and actions of allylglycine and glycine, it seems that these two substances may be reacting at the same membrane sites.

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Fast Presentation Rates and the Recall of Item and Order Information

SEVERAL investigators have suggested recently that there are two stages in short-term memory—a fast decaying buffer store and a more stable and organized store resulting from scanning, rehearsing and encoding operations[1-3]. In this context, rate of presentation should have two effects. Fast rates, for example, minimize the effective decay in the buffer store but at the same time reduce the opportunity for rehearsal. Posner[4] also suggests that increasing rate of presentation will show improved recall only in tasks which minimize the importance of retrieval strategies.

Although data on varying rate are sometimes inconsistent[5], it seems that sufficiently high rates (more than two items/s) produce increasing deterioration in recall[6]. Although these results seem to contradict the evidence for transient buffer storage, the latter has involved neither sequential inputs nor ordered recall.

We have attempted to clarify these issues by a theoretical and experimental distinction between order information, in which an item's serial position is stored, and item information. We suggest that the rehearsal possible at slow rates is largely used to organize order information, and that loss of order information leads to a deterioration in performance at high rates, when the traditional ordered recall is used.

Sequences of eight digits were prepared on 16 mm black-and-white movie film. Digits were drawn from random number tables, with two constraints: no two successive digits could be the same, and runs of digits in their correct numerical sequence were excluded. Each digit in a sequence occupied one frame of film only. By varying the number of blank frames between successive digits, the rates of presentation at a film speed of 24 frames/s were 12, 8, 6, 4, 2, $\frac{1}{2}$ and $\frac{1}{4}$ digits per second. Five different sequences of digits were prepared for each presentation rate, and were block randomized. Subjects were given all thirty-five sequences, followed by a short rest before repeating the same sequences a second time (there was no evidence to suggest learning of sequences).

Subjects performed two tasks. In the first, we required serial recall after each digit sequence, with subjects indicating omissions by blanks, and in the second, each sequence was followed by the auditory presentation of two probe digits. Subjects were asked whether each digit had occurred in the sequence. The four possible response alternatives, "yes, yes", "yes, no", "no, yes", "no, no", were arranged to be equiprobable and subjects were informed of this. The serial position of the probe digits was balanced for each rate. In this task the slowest rate was not used. Ten subjects, all university students, performed the two tasks in counterbalanced order.

In the serial recall task, responses were scored both for correct serial position and for item information. One mark was given for each digit recalled in its correct position. Item information was scored by giving one mark for every digit recalled correctly, irrespective of serial position. This gave a maximum possible score of 80 for each presentation rate for both methods. Scores for the ten subjects have been averaged and are shown in Fig. 1 together with standard deviations. Results of the probe digit task are also shown. In this case, we gave one mark for each correct response to a single digit. The maximum possible score was 20 for each presentation rate; again scores for all subjects have been averaged and converted to a percentage correct score for direct comparison on the same axes as the serial recall task. Only the slopes and not the absolute magnitude of the functions can be compared, because the performance measures are differently scaled.

The data for serial recall show that the amount of information recalled decays rapidly at presentation rates above ½ per second; this decay seems to be linear with respect to rate. The standard deviations indicate that variability is fairly constant, and does not seem to depend on rate or on the task. The data show clearly that subjects are able to retrieve digits more successfully than they are able to locate them in their correct serial position for all rates. Even at the fast rate, subjects are able to extract about four digits from the eight-digit sequence, but are only able to locate approximately one or two of these digits in their correct serial position.

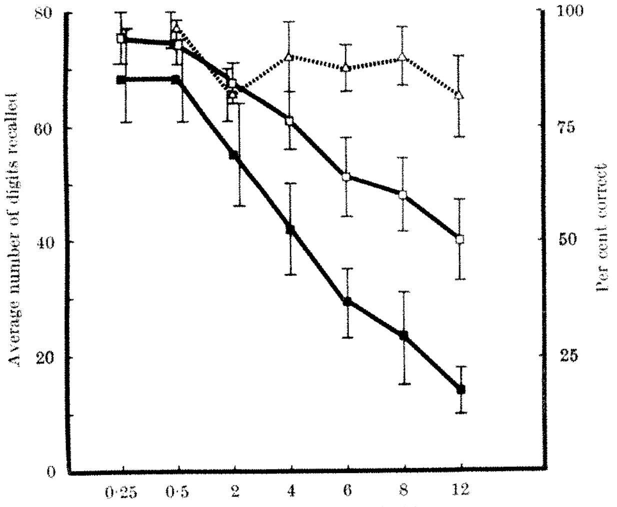

Fig. 1. Recall as a function of rate of presentation; probe recognition (△ - - - △), serial order recall (■—■) and serial order recall scored for items correct (□—□). Data points are means for ten subjects. Variability is indicated by a plot of one standard deviation. Where standard deviations overlap, those for the probe conditions have been displaced to the left and those for serial order recall displaced to the right for the purpose of clarity.

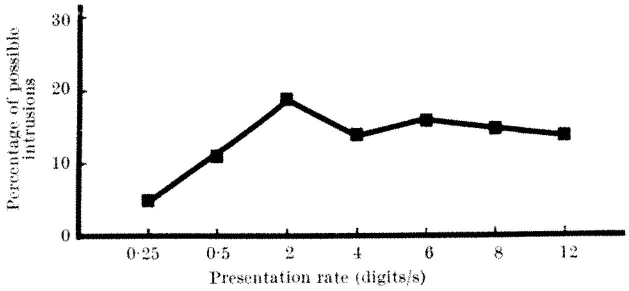

Fig. 2. Number of incorrect inclusions expressed as a percentage of total possible inclusions at each rate for the serial order recall condition.

This finding is further supported by data from the probe digit task. Ability to identify whether or not a digit was contained in a sequence does not seem to decrease systematically as presentation rate increases. At the fastest rate, subjects find no difficulty, and are able to give correct replies to 80 per cent of the probes. Errors to the probe given first (67) and given second (78) did not differ significantly (chi square = 0·84). The main feature of the functions is therefore their divergence with increasing rate. This divergence is evident both for the separation between the means and the standard deviations.

Because the serial recall task lends itself well to a guessing strategy, the component of chance must be isolated. The constraints placed on the randomization of the material make the calculation of a meaningful correction factor extremely difficult. Instead, we have taken, as a measure of guessing, the number of times a subject included in his response digits that were not present in the original sequence. The number of such intrusions made is shown in Fig. 2, expressed as a percentage of the total intrusions that were possible at each rate. Recall performance at the two slowest rates is about 90 per cent, which gives little opportunity to make intrusions. At the fast rates, the number of intrusions decreases slightly with speed of presentation, and the percentages are very low. Furthermore, we found that the number of omissions increases linearly with rate. This evidence seems to suggest that chance guessing is not a significant factor in the differential recall performance found at various rates.

The data for the probe task do not show any systematic deterioration of storage with rate, but suggest a more complex relationship between performance and rates. This is not reflected in the data for the serial recall task scored for content, because the task imposes retrieval constraints[4]. The serial recall data show that the retrieval of order information does depend on slow presentation and consequently on organizing effects of rehearsal. This is consistent with the evidence[7] that grouping preserves order and flattens the classical serial position *U*-curve and also with Neisser's argument[8] that rhythm is the natural carrier for serial order information. More generally, Henderson and Matthews[9] have suggested that rehearsal acts as an addressing system which plays an increasingly important part as retrieval constraints are increased, but which does not affect storage capacity. In this context, it is assumed that probe recognition involves minimal retrieval demands, while serial order recall involves maximal demands, and that serial recall of item information, or free recall, is located in between.

In the probe task, failure to find the improved storage which the buffer store hypothesis strictly requires can be accounted for in several ways. Delay in administering the probe digits adds a constant to retention time, which may mask the effect of increased rate; the fastest rates approach the limits of perceptual resolution and may lead to pre-mnemonic loss of input; and, finally, the diminishing effects of rehearsal with increased rate might be expected to obscure the concomitant increased utilization of buffer storage. These parameters are currently being investigated.

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Homoeologous Meiotic Chromosome Pairing in *Triticum aestivum* in which Chromosome 5B is replaced by an Alien Homoeologue

IN the hexaploid bread wheat (*Triticum aestivum* $2n = 6x = 42$) meiotic pairing is confined to fully homologous chromosome partners and inheritance is disomic. This arises from the activity of a single locus (*Ph*, *pairing homoeologous*) distally located on the long arm of chromosome *5B*[1,2]. In the absence of chromosome *5B*, pairing and recombination take place between homoeologous chromosomes, which are genetically related members of the three component genomes, as well as between homologues[3]. Knowledge of the *5B* system is of fundamental and practical significance. It throws light on the process of chromosome pairing during meiosis[4], and assists in the interpretation of the cytogenetic behaviour of polyploid wheat, and indeed of all allopolyploids. Moreover, interference with its activity has been exploited to cause abnormal recombination between homoeologues in order to introduce into wheat chromosomes useful genes from chromosomes of related species[5]. Consequently to extend the understanding of the system, as well as to facilitate its use in breeding work, attempts are continually being made to isolate new wheat genotypes displaying homoeologous pairing.

For this purpose we have been attempting to isolate wheat lines in which the *5B* pair is replaced by a pair of homoeologous chromosomes from a related species that does not have the genetic capacity to prevent the synapsis of homoeologues. The substitution of wheat chromosomes by alien homoeologues in this way involves a simple breeding procedure using as parents 41-chromosome wheat monosomics and 44-chromosome alien addition lines in which a single pair of alien chromosomes is added to the full wheat complement. When the monosomic chromosome in one parent and the alien chromosome in the other parent are genetically related homoeologues, plants can be isolated among derivatives of the breeding programme that are nullisomic for the wheat chromosome originally monosomic but still disomic for the alien chromosome. Such substitution lines often have phenotypes close to those of the original wheat parent[6].

In our attempts to replace chromosome *5B* by an alien homoeologue we used a chromosome of the diploid species *Aegilops umbellulata* ($2n = 14$). Because there is homoeologous pairing at meiosis in 27-chromosome *T. aestivum* × *Ae. umbellulata* hybrids deficient for chromosome *5B*, *Ae. umbellulata* does not carry out a genetic activity that prevents such pairing. Consequently the replacement of *5B* by its homoeologue from *Ae. umbellulata* would be expected to yield a genotype in which there is homoeologous pairing.

The separate disomic addition, to *T. aestivum* 'Chinese Spring', of six of the seven chromosomes of *Ae. umbellulata* was made at this Institute by Kimber[8], and we have subsequently added the seventh. The problem of recognizing the alien chromosome that relates to, and is homoeologous with, a particular homoeologous group of wheat chromosomes is simplified by the comparison of the phenotype of the alien addition line in which it is carried with those of tetrasomic plants of *T. aestivum*. The expectation is of similar phenotypic deviations from the euploid following increased dosage of any homoeologous group whether by tetrasomy or by alien disomic addition. On this principle, using the characters height, ear morphology, hairiness and susceptibility to powdery mildew, the chromosome of *Ae. umbellulata* designated *C* seemed likely to belong to homoeologous group 5. This likelihood was further increased by its morphological resemblance to the chromosomes of group 5. Like them, chromosome *C* has a submedian centromere (Fig. 1*b*). It also has a secondary constriction, delimiting a small satellite, as does chromosome *5D* in its original diploid source, although not in *T. aestivum* (Fig. 1*a*).

To substitute *Ae. umbellulata* chromosome *C* for *5B*, crosses were made between *T. aestivum* 'Chinese Spring' monosomic *5B* and the addition line with chromosome *C* added disomically to 'Chinese Spring'. Hybrids simultaneously monosomic for *5B* and *C* were isolated and self-pollinated and the resulting progeny were grown. In these progeny, the chromosome constitutions of fifty-six plants were checked, and of these three were substitutions with forty-two chromosomes and chromosome *C* disomic. Because of its distinctive small satellites chromosome *C* could be unequivocally distinguished, in somatic divisions, from all members of the wheat complement (Fig. 1*a*)

These disomic substitution plants and their sibs were grown in a glasshouse in continuous light and at a temperature of about 20° C, and their meiotic behaviour was examined at first metaphase. The substitution plants approximated to normal morphology and vigour, and indeed proved to be self-fertile, so that the allocation of chromosome *C* to group 5 was confirmed. The genome of *Ae. umbellulata* is designated C^u and so chromosome *C* will be referred to subsequently as $5C^u$ and the substitution line will be designated $5C^u/5B$ subst.

The other genotypes with which $5C^u/5B$ subst. was compared meiotically were all derived from 'Chinese Spring' and had the following chromosome constitutions. (1) Forty-three or forty-four chromosomes with monosomic or disomic additions of $5C^u$ to the full wheat complement ($5C^u$-mono-addn. and $5C^u$-diso-addn.) (2) Forty-two chromosomes with the normal wheat complement but *5B* and $5C^u$ both monosomic (*5B*-mono/$5C^u$-mono). (3) Forty-two chromosomes and the undisturbed wheat complement (euploid).

All the $5C^u/5B$ subst. plants had multivalents at meiosis and in the plant scored for Table 1 there was a mean of 0·76 multivalents per cell (Fig. 1*c*). Trivalents were more frequent than quadrivalents and there were also associations of five and six chromosomes. Chiasma frequency was low, relative to euploid, and this was reflected in the presence of univalents unrelated to the occurrence of odd numbered multivalents. This pattern of meiotic pairing is closely similar to that of plants of *T. aestivum* nullisomic for chromosome *5B* in which homoeologous pairing is known to occur[3,9]. Because there were multivalents resulting from homoeologous pairing, it can be assumed that some bivalents also formed between homoeo-

Table 1. MEAN CHROMOSOME PAIRING AT FIRST METAPHASE OF MEIOSIS IN *T. aestivum* WITH CHROMOSOME $5C^u$ SUBSTITUTED FOR $5B$ AND IN RELATED PLANTS

Genotype	Chromosome No.	Dosage $5C^u$	Dosage $5B$	Univalents	Mean pairing Bivalents	Mean pairing Trivalents	Mean pairing Quadrivalents	Mean chiasmata per potential bivalent*
$5C^u/5B$ subst.	42	2	0	1·57 (0–7)	18·83 (15–21)	0·40 (0–3)	0·30† (0–1)	1·82 ± 0·23
$5C^u$-mono-addn.	43	1	2	1·73 (1–5)	20·67 (19–21)	—	—	2·15 ± 0·11
$5C^u$-diso-addn.	44	2	2	0·07 (0–4)	21·97 (21–22)	—	—	2·27 ± 0·12
$5B$-mono/$5C^u$-mono	42	1	1	2·00 (2)	20·00 (20)	—	—	2·13 ± 0·15
Euploid	42	0	2	0·00 (0)	21·00 (21)	—	—	2·19 ± 0·11

Figures are for thirty cells per plant, with ranges given in parentheses.
* Potential bivalents; $5B$-mono/$5C^u$-mono = 20; $5C^u$-diso-addn. = 22; remaining genotypes = 21.
† 0·02 quinquevalents; 0·05 sexivalents.

logues. It can be concluded that, in the $5C^u/5B$ subst. condition, the effects of the deficiency of $5B$ are fully expressed and the presence of $5C^u$ does not compensate for the deficiency. On this evidence $5C^u$ is of null effect.

Reduced chiasma frequencies in association with homoeologous pairing, as in the present $5C^u/5B$ subst. plants, have been observed in other $5B$-deficient genotypes and ascribed to synaptic competition between homologues and homoeologues[10]. This competition reduces homologous pairing without guaranteeing homoeologous pairing.

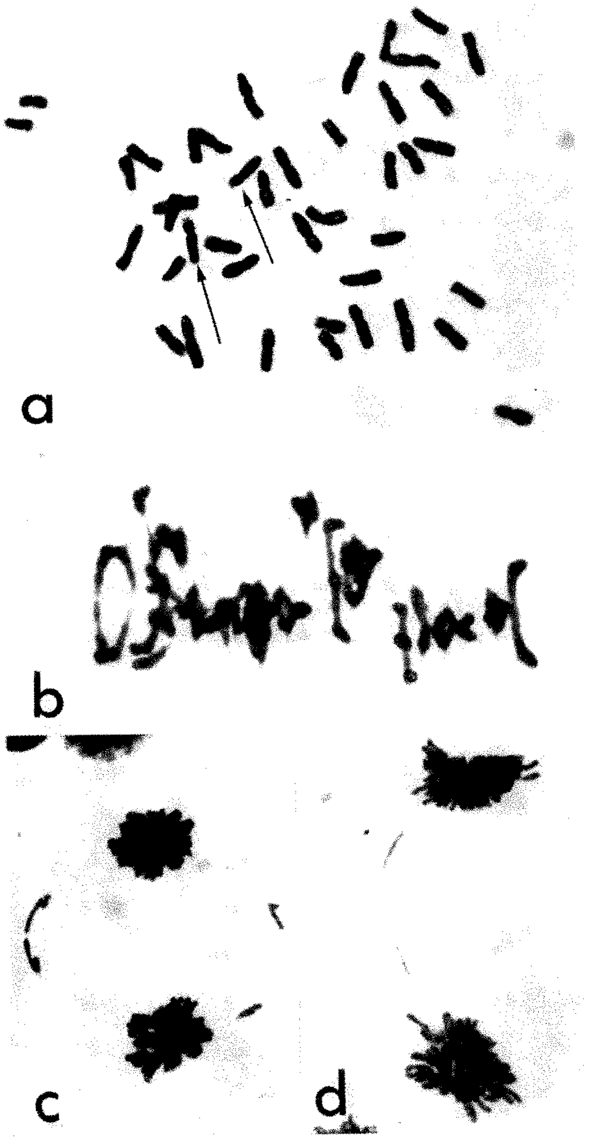

Fig. 1. *a*, Somatic chromosomes in a root tip squash, after pretreatment with *α* bromonaphthalene, of $5C^u/5B$ subst. with forty-two chromosomes and chromosome $5C^u$ (arrowed), with small satellites, disomic. *b*, First metaphase of meiosis in $5C^u/5B$ subst. with nineteen bivalents, and one quadrivalent on extreme left. *c*, First anaphase of meiosis in $5B$-mono/$5C^u$ mono with the monosomic chromosomes laggards and separating into chromatids, $5B$ on the left and $5C^u$ on the right. *d*, First anaphase of meiosis in $5C^u/5B$ subst. with an inversion bridge and fragment.

A further distinctive attribute of meiosis in the $5C^u/5B$ subst. plants was the presence of heterozygosity for a paracentric inversion, revealed by the occurrence of a dicentric bridge and acentric fragment in some first anaphases (Fig. 1*d*). There was no evidence of inversion heterozygosity in the sibs with only homologous pairing, and so it seems likely that the inversion differentiates homoeologous chromosomes and is thus apparent only when they synapse.

The $5C^u/5B$ substitution plants are vigorous, though short and with narrow leaves and stems, and lax spikes, compared with euploids. Furthermore, they are seed fertile and so are manageable as parents in breeding programmes. A progeny of twenty-three individuals derived from selfing a $5C^u/5B$ subst. parent was checked for chromosome constitutions, and twelve plants had forty-two chromosomes and were disomic $5C^u$, so retaining the parental constitution. The remainder had between forty and forty-three chromosomes, and all except two were disomic for $5C^u$. Consequently the $5C^u/5B$ subst. genotype is sufficiently stable for use in experimental and practical breeding work. Its value in the exploitation of homoeologous recombination in wheat breeding may be in providing a pollen-fertile $5B$-deficient condition without the complication of compensating aneuploidy for chromosome $5A$ or $5D$.

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Sunlight-induced Killing in *Saccharomyces cerevisiae*

ALTHOUGH ordinary sunlight has long been recognized as potentially deleterious to living organisms, little is known about the nature of genetic damage produced by sunlight or whether there are cellular mechanisms for removing the damage. The existence of cellular recovery systems (repair) has been demonstrated in a variety of organisms for the case of damage due to far ultraviolet light (< 2900 Å; designated here as UV), which is not, however, part of the sunlight spectrum at the Earth's surface (> 2900 Å)[1]. I have used two types of UV sensitive mutants of the yeast *Saccharomyces cerevisiae* to investigate (1) whether the mechanisms available for the repair of UV damage can also act on sunlight-induced lesions, (2) the extent to which sunlight exposure is potentially lethal, and (3) the nature of the lesions involved.

In what follows I use *uvs 9–3* to indicate the third identified mutant allele of the *uvs 9* locus, and *phr 1* for

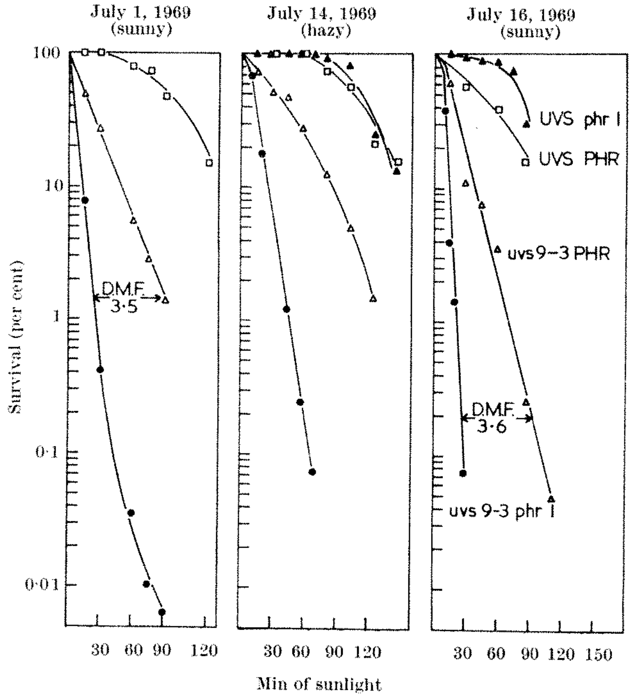

Fig. 1. The response to sunlight of wild type and radiation-sensitive strains of *Saccharomyces cerevisiae*: mutants at the *uvs 9* locus are sensitive to far UV and *phr 1* mutants are unable to photoreactivate damage induced by far UV. Experiments were performed in Mill Hill, London, at temperatures of 32°–34° C.

the first identified mutant allele of the *phr 1* locus. Capital letters signify the wild type. *uvs 9–3* mutants are considered[2] to be defective in excision-repair, a stepwise process of removal and replacement of damaged DNA[3–5], and *phr 1* mutants are incapable of photoreactivation[6], a process in which photoreactivating enzyme attaches to UV-induced pyrimidine dimers and splits them in the presence of visible light.

The UV and photoreactivation responses of the *uvs 9–3 phr 1*, *uvs 9–3 PHR*, *UVS phr 1* and *UVS PHR* strains used in this study have been described, as have been the methods of culturing cells and measuring survival[6]. Differences in survival between two conditions of irradiation or between strains are described by the dose modifying factor (DMF) which is the factor of increase in dose required to equate survival. Stationary phase cells were centrifuged, washed and plated after appropriate dilution to 1 per cent yeast extract, 2 per cent Bacto-peptone, 2 per cent dextrose and 2 per cent agar. Except for the controls, which were placed in aluminium foil, the Petri dishes were exposed to sunlight with lids removed. Experiments were performed on three different days beginning at approximately noon; two were sunny and one was hazy. The total sunlight exposure has been corrected for changes in the dose rate by measuring survival of the *uvs 9–3 phr 1* strain at succeeding 15 min intervals and comparing this survival with that in the first 15 min. Temperatures ranged from 32°–34° C.

The effects of sunlight can be very profound in the absence of genetic repair, as shown in Fig. 1. On a sunny day survival of the *uvs 9–3 phr 1* strain is less than 1 per cent after only 30 min exposure while in the *UVS* strains it is approximately 100 per cent. A major category of lesions seems to be pyrimidine dimers, as shown by the better survival (DMF: 3·5–3·6) of the *uvs 9–3 PHR* strain (which contains photoreactivating enzyme) than of the *uvs 9–3 phr 1* strain (which does not). As for the case of UV-induced killing, photoreactivation of sunlight-induced damage is not 100 per cent effective, so that lesions other than dimers may be involved. The presence or absence of photoreactivating enzyme in the *UVS* strains does not increase survival; this is rather surprising, for after UV treatment of a *UVS* strain the damage is photoreactivable at all levels of lethality. It would thus seem that the dark repair mechanism leaves no damage to be photoreactivated, perhaps because of a relatively slow exposure rate which allows more time for dark repair of pyrimidine dimers. The ability to photoreactivate pyrimidine dimers could be relevant, therefore, in conditions of growth (that is, log phase) which do not leave enough time for dark repair. Although dark repair seems to be nearly 100 per cent effective against lesions that arise during short exposures to sunlight, killing does occur at higher doses. Following 30–60 min of sunlight, significant lethality is detected, and after 2 h nearly 80 per cent of the cells are dead.

The observation that survival characteristics for all the strains are similar on both sunny and hazy days suggests that there is little attenuation of the component of sunlight that can cause killing. The DMF for a hazy against a sunny day is approximately 2, as determined from the survival of the *uvs 9–3 phr 1* strain on the two types of days.

Short exposures to sunlight thus seem potentially very lethal for yeast cells. Harm has reported similar results for bacteriophage and bacteria[7] and has suggested that UV-sensitive bacteria be used as biological dosimeters for sunlight[8]. Because death in yeast can be considerably decreased by photoreactivation and by what is assumed to be excision-repair[6], both of which are known to act on damaged DNA, I conclude that sunlight-induced lesions occurring at low doses are present primarily in DNA. The effect on the *UVS* strains at high doses may result from (1) a saturation of the repair processes—unlikely because the exposure rate is so low, (2) an accumulation of non-repairable DNA damage, or (3) the production of non-DNA lesions, as indicated by the significant killing observed[9] in bacteria at wavelengths greater than 3100 Å. If (2) or (3) is true, the damage can only be expressed when it has accumulated to a particular level because little or no killing is observed at low doses.

The role of repair mechanisms in removing sunlight-induced lethal damage has important human implications. Cleaver[10] has presented evidence suggesting that dark repair occurs in normal human cell lines but is absent in cells from patients having xeroderma pigmentosum, a genetic disease characterized by susceptibility to skin cancer after short exposures to sunlight. Results in the present experiments indicate that xeroderma pigmentosum symptoms may be caused by an extensive amount of sunlight-induced lesions in dermal cells which, in normal cells, would be removed by dark repair processes.

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Book Reviews

SOCIAL SCIENCE

Behavioral Sociology

The Experimental Analysis of Social Process. By Robert L. Burgess and Don Bushell, jun. Pp. xiii + 418. (Columbia University Press: New York and London, February 1970.) $12.50; 113*s*.

FROM time to time impatience with the "non-scientific" qualitative nature of social science inspires an attempt to develop a rigorous quantified experimental "behavioural" science with its roots in experimental psychology or even physiology. The most notorious of these is the attempt by the Pavlovians to explain all behaviour as a chain of conditioned reflexes. The crude behaviourism of the 1920s and '30s followed this trend, and its excesses were partly responsible for the counter-revolution of the gestalt psychologists and their more modern successors in the field of social psychology. It is the misfortune of such attempts to lead to scientism rather than science and to the reductionism of what Viktor Frankl has described as the "terrible generalisateur (who) turns biology into biologism, sociology into sociologism, and psychology into psychologism"[1].

At first sight this book looks like yet another assault on the sociological citadel in the name of a new behaviourism. But a robust assertion by Homans sets a more reasonable aim:

> "Let us not worry about whether these are sciences at all. Any activity that tries to state relationships between properties of nature, whether or not the statements are exact or general, whether or not they make predictions possible, so long as the test of the truth of the statement lies in the data themselves, is a scientific activity, and by this standard much of the activity of all the social sciences is certainly scientific" (page 1, prologue).

He claims that the ultimate importance of the propositions of behavioural psychology is that they stand as the most general propositions of social science. They do not involve reducing all explanation of behaviour to the behaviour of the isolated individual, still less to concern with individual differences where the interest is in statistical regularities in the behaviour of large groups or classes of individuals. Although he claims his stand is "reductionism", the position he, and the other authors, adopt is not dissimilar to that of Gluckman and Devons[2] to whom reductionism is anathema:

> ". . . some psychologists criticize economics because of its simple and crude assumptions about human motivation; and some psychologists criticize social anthropology because it neglects the complexity of the structure of the personality. But the economist or anthropologist may be making these neglectful assumptions knowingly and deliberately. We argue that the issue is not whether the assumptions are crude, but rather whether they materially affect the analysis and conclusions of economist and anthropologist".

The behaviourism on which the authors draw is the kind familiar in what is now described as behaviour-therapy and derives from the work, largely associated with the name of B. F. Skinner, on "operant" conditioning. This is one of those terms which look more precise than they are. For example, on page 27, operant behaviour is defined as "the behavior of individuals that operates on the environment in such a way as to produce some consequence or change in it which, in turn, modifies subsequent performances of that behavior".

The order of the chapters in the book is designed to alternate theoretical analysis with experimental data. As the latter are by different hands at different times, the strain on the editing proves too great. Thus, for example, the story of the "shaping" of the social behaviour of 13 year old Justin is mentioned in two successive chapters (on p. 281 and p. 304) without any cross-reference. The experimental chapters consist of two kinds of demonstrations: first, that social aspects of behaviour can be "shaped" by means of "schedules of reinforcement", often without the knowledge of the subject(s) that this was being done; and second, that undesirable behaviour of a pathological or criminal nature can be eliminated or greatly reduced by carefully controlled operant conditioning. Perhaps the most advanced of these examples is that which demonstrates how a subject can learn to imitate the general behaviour of a model, not just the specific imitating behaviour that is being reinforced.

Many of these experimental data will prove surprising to nobody and familiar to those who have read Eysenck. That children treated firmly and rewarded for doing what is required, and punished by being removed from the scene and ignored for doing what is to be discouraged, will learn to behave better sounds pretty obvious; what is truly revealing is the realization that our usual methods of treating bad behaviour sometimes reinforce just the reverse of what we intend.

The most important chapter is that by Burgess and Bushell on "Behavioral View of Some Sociological Concepts". Their starting point (p. 274) is given thus:

> "The experimental psychologists who have developed the principles of behavior did so in a highly controlled environment. Within this setting they confined their observations to simple arbitrary responses which were chosen for reasons of experimental ease rather than social significance. Only recently have attempts been made to investigate systematically the application of these principles to the analysis of social behavior".

They then discuss a number of critical sociological concepts. Their discussion of socialization is particularly valuable and, pointing to the important role of the primary group, they observe, "The shaping requirement for intense one-to-one interaction is met more effectively by the primary group arrangement than by any other frequently encountered social device". Acquisition of values by socialization "combines the principles of shaping, the development of conditioned reinforcers, and the graduation from high-density reinforcement schedules to highly intermittent ones". With a surprising degree of success they apply the same approach to role behaviour, verbal behaviour, the concept of the "self", and to the acceptance of behaviour models.

This approach proves less convincing in the chapter by Kunkel on "Behavioral Aspects of Social Change and Economic Development" and in Richard Emerson's chapter on "Operant Psychology and Exchange Theory". Nevertheless, these offer some interesting applications. But Emerson must have the last word:

> "In branching out from operant psychology, this theory has been highly selective and, in large part, arbitrarily so. For example, negative reinforcement (and "aversive control" in exchange relations) has been virtually ignored, despite its obvious importance in social affairs. There is much more available in operant psychology to be drawn upon. But, even more to the point, *this theory* (so far) *has been narrowly selective in choosing operant psychology as its sole psychological base.* Not all psychological knowledge is contained within the operant framework, and the strategy put forth here is free to draw upon reliable and relevant principles wherever they can be found" (p. 404).

What we have then is not a replacement of sociology with a new outgrowth of operant behaviourism, but a solid approach to the underpinning of sociology with a widely based experimental psychology. A. B. CHERNS

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ARCHAEOLOGY OF THE FUTURE?

Science in Archaeology

A Survey of Progress and Research. Revised and enlarged edition. Edited by Don Brothwell and Eric Higgs. Pp. 720+40 plates. (Thames and Hudson: London, February 1970.) 147*s* boards; 42*s* paper.

THE publication of a second edition of *Science in Archaeology* only six years after the first edition emphasizes two things: the enormous steps that have been taken in recent years in applying a much wider range of scientific techniques and approaches to the needs of archaeology (720 pages compared with 595) and a slight, but noticeable, change of emphasis. Thus the differences between the two editions are significant because they point to the direction along which the subject is at present developing. Physical methods of dating and prospecting, after a rapid development in the 1950s and early 1960s, now seem temporarily to have reached a slower rate of progress. More has been added on the potassium–argon method and a potentially useful new approach using a fission track technique is summarized, but there the matter rests. Similarly in the fields of microscopy, radiography and the general physical characterization of artefacts, little has been added, apart from a survey of recent work on obsidian and two very useful articles on the use of the scanning electron microscope and the application of X-rays.

One of the most impressive growth points in the subject in recent years has been in the field of statistics and computerization. Innumerable articles have been written attempting, with varying degrees of success, to apply statistical techniques to sometimes rather intransigent archaeological data. The more thoughtful writers have usually preached optimistic caution; the less thoughtful critics have heaped condemnation. Now for the first time we are offered a balanced 44-page consideration of the whole field, written, significantly perhaps, by four authors from the viewpoints of archaeology, anthropology and zoology. The range of approach is wide and sometimes necessarily superficial, but the text is well supplied with pertinent archaeological examples. As an introduction to the present state of knowledge for less mathematically minded archaeologists, this is essential reading.

The second area of rapid development is in the biological application to (or, more strictly, of) archaeology. Mollusca of various kinds are now considered in some detail and there is a very welcome discussion of the problems posed by early animal husbandry. Surprisingly, however, the process involved in the early "domestication" of plants tends to be sketchily treated, and this at a time when so much new work is under way. Valuable though these twenty-three contributions on plants and animals are individually, and many of them are exceptionally good, the overall impression they give is of a fragmentation in approach and interest, when, as the editors point out in their stimulating introductory chapter, the present emphasis should be on the very close integration of archaeology and the biological sciences. The one article to get close to this ideal is a thought-provoking treatment of social organization as a population regulator.

On style and presentation the book is difficult to fault, and the editors have managed to overcome most of the difficulties inherent in producing a compilation of sixty-one contributions. One omission, however, which should be put right in later editions, is the absence of the dating of the individual articles. At a time of such rapid advance it is essential to know when the latest revision of each was carried out.

This is, without doubt, an important work which must be read by all archaeologists and all interested in the archaeology of today and the future. Scientific approaches and techniques are now a part of the substance of the discipline, and only by accepting and building on this premise can there be progress. The diehards of the old school must now sound their final retreat.

Finally the price: seven guineas is a fair price for the hardback, and the publishers will be blessed by generations of students for producing a paperback version for as little as 42 shillings. BARRY CUNLIFFE

PREHISTORIC MEDICINE

Palaeopathology

Diseases and Injuries of Prehistoric Man. By Paul A. Janssens. Pp. xiii + 170 + 75 plates. (John Baker: London, February 1970.) 55*s*.

THE subtitle of this book is a misnomer; almost as much attention is devoted to the diseases and injuries of fossil animals as to those of man. The work is divided into twenty-seven chapters varying greatly in length, content and importance, and bearing titles which in many cases have little connexion with the matter discussed in them.

The author first attempts to define medicine as any action carried out to safeguard man against disease, and finds in it what he terms a science-factor and a law-factor, the latter necessary not only to protect the sick individual but also to protect knowledge. A brief discussion of climate, economy and environment follows. After some consideration of the limitations imposed upon the palaeopathologist by the nature of the material at his disposal he provides a useful but too brief summary of the effects of cremation upon bone. His study of traumata affords him an opportunity to speculate upon the social relationships of early man. A long chapter on palaeolithic art seems to have been included chiefly to prove that prehistoric man had some knowledge of animal anatomy—a not improbable assumption. The discussion of the strange symbols often found on the walls of caves in association with pictures of animals is stimulating and suggestive. From a review of some modern folklore the author derives hypotheses concerning the reason for the frequency with which fractured calvaria are found at ancient burial sites. A short section on anomalies of development and their representation in Egyptian paintings is followed by a more extended consideration of Venus figurines which for the most part exhibit steatopygia. He holds that an important factor in their interpretation is the average duration of life at the time and goes on to present some evidence concerning this average, but omits to demonstrate any connexion between this and the figurines in any explicit way. A reasonably comprehensive account of the occurrence of tumours in man and animals is followed by a sketchy explanation of the way in which the body reacts to infection and a long chapter on arthritis. The author seems to assume a bacterial basis for most arthritic conditions and claims that bacteria became pathogenic only with the appearance of the vertebrates. (The most likely explanation of this improbable claim would be the misreading of a sentence in Simpson's book *Life of the Past*.) Periodontitis, tuberculosis, syphilis and some other infections are briefly considered; in the case of the last the author, after some hesitation, accepts the view that it was brought to Europe from America in the 15th century. He then deals at some length with the outlines of mutilated hands which appear on the walls of some caves and, rejecting all other suggestions, he explains them as amputations effected therapeutically to arrest gangrene following paronychia or Raynaud's disease.

The longest chapter concerns itself with trepanation and the syncytial Y. He holds that trepanation had no ritual significance (except possibly in the Loire region) but had invariably a therapeutic objective. He finds some interesting parallels to the syncytial Y among living peoples.

The book is profusely illustrated, but some plates add little to the text. The work ends with a glossary and a bibliography. The latter gives a useful list of French and Spanish work in this field; the former arouses curiosity about the readers for whom the book was written. Many names familiar to anybody with an elementary knowledge of biology are glossed; for example, humerus, femur. Others in everyday use in the medical world are glossed, often inaccurately; for example, glioma—a swelling of the nerves! Pathological conditions are given names never used in medical works in English; for example, paranitium for paronychia. The translator would seem to be unfamiliar with much of the English medical vocabulary.

Nevertheless, in spite of many shortcomings the work contains much information, especially concerning the author's own observations and conclusions. Its chief defects are its superficial treatment of many topics, its lack of order and critical judgment. It omits any detailed consideration of the confusion caused by the post mortem action of environmental factors on human (and animal) remains and makes no suggestions about topics the further study of which might be most productive. As a serious study of a subject which is attracting increasing attention the book is disappointing. H. HUGHES

EARLY BEHAVIOUR

Brain and Early Behaviour

Development in the Foetus and Infant. Edited by R. J. Robinson. (Centre for Advanced Study in the Developmental Sciences.) Pp. xvi + 374. (Academic Press: London and New York, January 1970.) 100*s*; $15.

THIS book is a collection of nineteen papers from a study group on the brain mechanisms behind early behavioural development in the foetus and newborn infant, under the aegis of the Centre for Advanced Study in the Developmental Sciences being established at Minster Lovell near Oxford.

It was a multidisciplinary group, which included Heinz Prechtl of Groningen speaking on the brain and behaviour mechanisms of the newborn; Bergström of Helsinki on electrical parameters of the brain during ontogeny; Humphrey of Alabama on the neuroanatomical basis for postnatal repetition of human prenatal activity sequences; Schulte of Göttingen on nerve conduction velocity in relation to myelination and maturity; Wolff of Boston on motor development; Dittrichova of Prague and Petre-Quadens of Antwerp on cerebral sleep patterns; Bronson of California and Bower of Harvard on the development of vision; Lipsitt of Rhode Island and Papousek of Prague on learning and conditioning in the foetus; Ploog of Munich on primate studies; Sander of Boston on the organization of behaviour; and Robinson of London on cerebral hemisphere function in the newborn. Seventy-five pages are occupied by discussion after the papers. As usual with published symposia, the discussions are difficult to read, but much interesting material would have been lost if they had been omitted. The book is intended to analyse early behaviour from the viewpoint of underlying brain mechanisms, and is expected by Robinson, who edited the book, to interest neurologists, paediatricians, psychologists and biologists.

The section on the smile of the baby in his first six weeks revealed the usual confusion about its interpretation and significance. When it seemed to be suggested that the baby's smile in response to the mother's overtures, which include visual, kinetic and auditory stimuli, need not be distinguished from facial movements during sleep, Heinz Prechtl aptly asked what the sleeping baby was likely to be dreaming about. As always with these psychological studies, no mention was made of the relationship between the age of smiling and the maturity (and perhaps the personality) of the infant. After all, any paediatrician interested in the development of the infant knows that all mentally defective infants, such as mongols, are later than the average baby in beginning to smile; it would not be surprising if an infant with a high developmental potential were to begin to smile earlier than usual. I have personally seen three such infants who smiled in response to maternal overtures within four days of birth, and who a few days later were vocalizing well in association with the smile. One might add that, as always with these studies, there was no mention of the developmental features which precede the smile—the baby's intent regard of the mother as she speaks to him, his opening and closing of the mouth, his bobbing of the head up and down—until gradually the real smile becomes obvious.

I feel confident that the book will achieve its object. It is bound to be of interest to the neurophysiologist and to those paediatricians who are interested in developmental neurophysiology and in the application of the sophisticated apparatus and the computer so necessary for these studies. It would have been a pity if all the interesting information and provocative thoughts in it had not been made available to those not able to attend the meeting. I am glad to have the book in my departmental library. R. S. ILLINGWORTH

THE MEGALOBLAST

The Megaloblastic Anaemias

By I. Chanarin. Pp. xv + 1,000. (Blackwell (Scientific): Oxford and Edinburgh, 1969.) 200*s*.

THOUGH preceded by the classic clinical papers of Combe, Addison and Biermer, Ehrlich's description of the megaloblast in 1880 was the first definitive pointer to the complex of diseases so ably marshalled by Dr Chanarin. Some idea of the magnitude of his undertaking may be gauged by the references, which total more than three thousand. It will therefore be appreciated that this is a tremendous source of information, dealing in its thirty-six chapters with the basic chemistry, nutritional aspects and metabolism of cobalamins and folates, the physiological and morphological effects of lack of these substances in man and other organisms, and the various clinical disorders associated with megaloblastic anaemia. Such diversification as illustrated by this book highlights the dilemma of the modern haematologist who must range over so many disciplines that he is in danger of becoming a jack-of-all-trades; but Chanarin has mastered the multidisciplinary approach and is particularly well qualified to do so in the sections dealing with folate metabolism, the FIGLU test and the estimation and function of intrinsic factor, in all of which he has made important contributions. This is the first major attempt to synthesize a detailed and comprehensive account of the megaloblastic anaemias and it is remarkable that it has been accomplished by one author.

The diagrams and illustrations are on the whole of high quality, but the megaloblasts facing pp. 352 and 358 do not have the impact of colour—essential for finer diagnostic distinctions. The erythroblasts opposite p. 370 would not be accepted as megaloblastic by every haematologist. One wonders whether the figure on p. 460 (racial distribution of pernicious anaemia) is relevant enough for inclusion, particularly because it is partly contradicted by more valid data in the following pages. A final point of criticism is of the thread of argument on pp. 818 and 819, concerning the cause and the diagnosis of megaloblastic anaemia in pregnancy; somebody familiar with this problem can follow the text, but to the uninitiated the presentation might seem contradictory and the argument circular. These points are minor, as are also the extremely few bibliographic errors. The general format and printing are of the usual high Blackwell standard and the price is reasonable.

There is much here for the biochemist, biologist and nutritionist, but the chief appeal of this major work of reference is to the medical profession, in the clinic as well as the laboratory. Anybody doing research on megaloblastic anaemia or studying for a higher qualification in haematology will find this book essential reading and it should be in every department of haematology.

H. B. GOODALL

DEFENSIVE NETWORKS

The Reticulo-endothelial System

By A. E. Stuart. Pp. xi + 255. (Livingstone: Edinburgh and London, 1970.) 120*s*.

THIS book is the first general account in the English language of the system of cells throughout the body which plays an important part in defence. It is written by a pathologist who has made important contributions to knowledge of this system, known as the reticulo-endothelial system (RES).

The author starts by describing in some detail the anatomy of the RES; the section on pulmonary macrophages is particularly good. Next, there is a brief but intelligible account of the handling of antigens and iron, followed by an outstanding review of work on stimulation and depression of the RES, which gives special attention to the effects of lipids, and then there is a brief note on the induction of vascular disease. The chapter on cellular immunity in bacterial infections is of an equally high standard.

There follow adequate descriptions of intracellular killing of bacteria, and of the reaction of the system to virus infection. In his account of the macrophage in culture, the author has chosen to be rather selective; much of the work described is his own. This is perhaps the only way of producing a readable account of such a diffuse field. The apparent equation of the extended macrophage in tissue culture with the dendritic macrophage *in vivo* is at present an assumption.

Next there is a slightly scrappy summary of a very scrappy subject—the response of the RES to cancer and grafting. The last chapter is a concise account of the comparative anatomy of the RES, with a memorable picture of a contemplative octopus. This chapter also includes a short consideration of discrimination by macrophages.

In a day when savants spend their days counting the number of pico-devils dancing on the end of a microvillus, the catholicity of Dr Stuart's learning is impressive. It is a considerable achievement to have written a book which passes from the post-mortem room, by way of carbon clearance tests and some ultrastructure, to the octopus and the crayfish, and yet which remains a cohesive whole. A single-author book covering such an extent must, as here, vary in depth of treatment. The style is clear and readable, sometimes picturesque. Cilia "beat with purposeful strokes"—of course they do. The pictures are numerous, mostly good and often coloured, and the bibliography is extensive. The production is good and the price is probably fair.

I enjoyed reading this excellent and authoritative text, and can recommend it warmly to honours students in the medical and biological sciences, to postgraduate students, and to pathologists.

IAN CARR

PHYSIOLOGY OF FISHES

Fish Physiology

Edited by W. S. Hoar and D. J. Randall. Vol. 1: Excretion, Ionic Regulation and Metabolism. Pp. xiv + 465. Vol. 2: The Endocrine System. Pp. xiv + 446. (Academic Press, New York and London, November and December, 1969.) 215*s* each.

FOR one whose first ideas about comparative physiology were stimulated by Hogben's book of that title during the twenties, it is both amazing and delightful to see, reviewed in these books, something of what has been achieved during forty years, just within one group, even one as diverse as the fishes. It is almost equally amazing to see what has been done in the much shorter time since Dr Margaret Varley edited *The Physiology of Fishes*.

Some of the more recent work will have been stimulated by Dr Varley's book, as a convenient reference and review, just as this new series will stimulate students and more senior workers during the next ten years. The subject is, however, intrinsically fascinating, if only because of the diversification of fish species over almost the whole range of aquatic habitats, and the interest of biologists of many kinds seems assured. More specific will be the debt of fisheries research workers, although one would like to think that to some extent this has been paid in advance by that same process of stimulation, and it is good to see among the list of authors already identified several who have worked or still work in recognized marine and fisheries research laboratories, or who have had definitive connexions with them. Fisheries research has achieved more than is often realized, perhaps, but it is necessarily a slow business, and it was perhaps especially slow in realizing the necessity for understanding fish behaviour and its physiological basis. Recently, some useful advances have been made by fisheries scientists in both, but there is far to go and progress must depend greatly on the work of academic colleagues. It is good that this series of volumes, practically all of which promise to be relevant in fisheries terms, is intended to include a section on behaviour itself.

The first volume is devoted to aspects of metabolism: ionic regulation and osmosis, excretion, intermediary metabolism, nutrition and energy requirements, and the like. There is a full account of the physiology of the lower vertebrate kidney, with an introductory chapter on the body compartments and the distribution of electrolytes, and smaller sections on the effects of salinity and on salt secretion. The second volume is devoted to endocrinology although, in spite of the reasoned apology, it will surprise many that the endocrinology of the gonad has been deferred for volume three (and of the pineal gland for volume four) if only because of the known interrelations of the endocrine system. There is a major section on the pituitary, including a separate chapter on the neurohypophysis, with smaller sections on prolactin, the endocrine pancreas, the caudal neurosecretory system and the ultimo-branchial glands, together with thyroid control and the adrenal cortex (although not the medulla). While noting the author's view that an exhaustive review of thyroid function was neither possible nor desirable, it is also noteworthy that reference is made here (and elsewhere) to early literature on chemical identification of hormones with little or no evaluation of the earlier test procedures adopted, although there is at least an acknowledgment in the chapter on the adrenal cortex of the considerable analytical developments of the past decade.

As a whole, however, these first two volumes are admirable, and promise greatly for the remainder, as one would expect of the editors. They have set out their hope that this series of six volumes "will prove as valuable in fisheries research laboratories as in university reference libraries and that it will be a rich source of detailed information for the comparative physiologist and the zoologist as well as the specialist in fish physiology". I think it will, and I expect that it will be found in all relevant libraries, in spite of the heavy price that has to be paid.

C. E. LUCAS

NEW WORLD FLORA

Vascular Plants of the Pacific Northwest

Part 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. By C. Leo Hitchcock, Arthur Cronquist, Marion Ownbey and J. W. Thompson. Pp. 914. (University of Washington Press: Seattle and London, November 1969.) 238*s*; $25.

THE recent publication of part one now completes this magnificent and already well known flora, the other four parts (each worthy of being called a volume) having appeared in reverse order during the past fifteen years, starting with Cronquist's Compositae.

The Pacific North-West, as defined by the authors of the flora, is an extensive area of territory covering the states of Washington, northern Oregon, Idaho north of the Snake River plains, the western mountainous part of Montana and southern British Columbia. Some 4,000 species of vascular plants are known to be indigenous here or to have been introduced and these have now been fully dealt with in the five parts. As the title indicates, part one includes the vascular cryptogams (ferns and fern allies), gymnosperms and monocotyledons, most of them dealt with by the senior author C. L. Hitchcock. Users of this and probably the other parts of the flora may possibly be confused by the circumscription and nomenclature of the divisions and classes of the higher plants. For instance, here the name Pinophyta is used for Gymnospermae, Magnoliophyta for Angiospermae and Liliatae for Monocotyledonae, following the recent proposals of Cronquist *et al.* in *Taxon*, **15**, 1966. The sequence of monocotyledon families also follows Cronquist's *Evolution and Classification of Flowering Plants* (1968) and might seem strange to those not already familiar with this treatise. Besides the usual keys to families, genera and species and rather full descriptions, complete "regional" synonymy is given together with details of chromosome number (very frequently known), "genuine" vernacular names and information on distribution and ecology. Finally, notes on infraspecific variation have been added. Following the (unfortunate ?) tradition of most temperate floras, no matter how elaborate, no specimens are, however, cited other than type material.

Perhaps two features above all contribute to the usefulness of this particular part. The first of these is the excellence of the illustrations by J. R. Janish. Practically every species has been illustrated by simple but extremely clear and accurate line drawings, depicting habit and diagnostic characteristics. The second is the inclusion of special keys. One to the grasses of the area (again illustrated) is based purely on vegetative features and will be of great potential use to pasture ecologists and agriculturists who so often must, of necessity, identify sterile material. There is also a key to aquatic plants (all vascular groups included), again chiefly on vegetative characters.

This volume, being the last and largest part of the flora to appear, includes several sections relating to the work as a whole. There is, for instance, an index to all the plant families, a glossary and a corrigendum to the other parts. Finally, there is a complete index to vernacular, generic and specific names.

The sheer bulk of this flora means that it will serve chiefly as a work of reference, but the thoroughness of its production will certainly ensure for it a status and value far above that of a mere regional flora, for many years to come.

BRIAN T. STYLES

QUANTUM CHEMISTRY

Methods of Molecular Quantum Mechanics

By R. McWeeny and B. T. Sutcliffe. (Theoretical Chemistry—A Series of Monographs, Vol. 2.) Pp. ix+307. (Academic Press: London and New York, December 1969.) 84*s*; $13·50.

WRITTEN for a far more sophisticated and exacting readership than the first volume in the series, this is a book in which even the most experienced quantum chemist should find a great deal of value. A rigorous yet economical exposition, characterized throughout by refreshing critical insight, it has all the qualities one would expect of its authors.

Being designed "for students specializing in theoretical chemistry or molecular physics", it uses techniques which go well beyond the repertoire of the average chemist. It is certainly a work of consolidation rather than primary instruction. At first sight, much of the analysis will seem formidable to student readers; and, while those who do battle with the difficulties will find themselves very well rewarded, I think this book will probably be more popular with teachers of quantum chemistry than with their students.

Together with two appendices, the first two chapters present a brisk survey of the general principles and theorems of wave mechanics, illustrated by reference to one- and two-electron systems. Next comes a careful account of the various operators used in formulating many-electron antisymmetrized wave functions.

Having completed the usual preliminaries (though by no means always in the usual way), the authors then provide a most welcome discussion of electronic charge distribution, including, of course, a description of density matrices.

The next three chapters deal in turn with the Hartree–Fock theory and its principal elaborations, with valence–bond theory, and with various recent developments (generalized product functions; variation–perturbation theory for group functions; cluster development; correlated pair functions).

A chapter on electrical and magnetic effects is supplemented by a quite substantial appendix dealing with relativistic terms in the Hamiltonian operator.

The book concludes with a description of some typical molecular calculations. This is the only chapter in which any numerical results are discussed.

It could perhaps be argued that much of the material in this book could have been expounded accurately and effectively in a simpler way; but this was clearly not the authors' intention, and they are to be congratulated on producing a contribution to the didactic literature of quantum chemistry that is really distinctive.

E. THEAL STEWART

Obituaries

Dr Peyton Rous

EXPERIMENTAL pathology and cancer research, and indeed the whole of biological and medical science, have sustained immense loss from the death, on February 16, 1970, of Francis Peyton Rous, at the age of 90. His special fields will never be the same without him. Yet our main emotion must be of gratitude for his long life and epochal contribution.

Rous's mother was descended from Huguenots who had settled in Virginia after the Edict of Nantes; and his father was a Baltimorean of English forebears. Reading of his early life evokes and recaptures in remarkable degree the spirit of a great period in the history of the United States and, a little later, that of an outstanding phase in American medicine during the early years of the century. After graduating MD at the Johns Hopkins Medical School in 1905, Rous's interests very early turned to research, with a period at Ann Arbor under Warthin and later in the Rockefeller Institute, New York, where he spent the great part of his working life in the most happy and congenial of conditions. His move to the Rockefeller was a stroke of genius on the part of Simon Flexner, who accorded him complete freedom and every encouragement, a privilege which Rous thenceforth ever valued and later recorded on many occasions.

Between 1907 and 1910 Rous made a wonderful start, with papers on lymphocyte physiology, haematology, the cerebrospinal fluid and tissue transplantation. Very soon there came the stroke of fate. In the words of O. H. Robertson, "One day a chicken fancier came in to Rous's laboratory bringing a fowl with a large lump on its leg. Concerned for his flock, this man had taken the fowl to several unresponsive pathologists. Rous saw that the lump was a tumour and in a class of animals unstudied in such relation". The tumour proved to be a spindle-cell sarcoma, which Rous was able to transmit to other fowls of the same inbred stock, not only by grafting but also through the injection of cell-free filtrates. The times must have been propitious, following Ellermann and Bang's description of an apparent leukaemia virus in 1908; yet Rous's discovery was a monumental and key event in the history of cancer research.

The Rous agent, as it came to be called, also seemed to be viral in nature, with extraordinary specificity in transforming normal cells into the exact replica of the sarcoma cells from which it was derived. Later, Rous and his co-workers uncovered other avian oncogenic viruses, each again with its own individual transforming property. Especially with J. B. Murphy, Rous studied the growth of such tumours and viruses in the avian embryo, perhaps the earliest use of this technique, and an example of the freshness and originality which characterized so much of his work and still exert their influence.

There is evidence that these discoveries were not perhaps fully appreciated at the time, as was seen from a species of metaphysical pathology on the part of the orthodox, according to which the tumours, although sarcomata, could not be tumours since they were virus-induced. Rous was also disappointed by his failure, after strenuous efforts, to demonstrate any kind of viral transmission of cancer in mammals. For some fifteen years he then spent his energies in more physiological pursuits, and this period embraced a wide variety of classical papers. Outstanding topics were the preservation and transfusion of blood, with practical application in both the First and Second World Wars by means of the Rous–Turner formula; the genesis of gallstones and the relation of blood destruction to bile pigment output; liver function in many aspects; and the measurement of hydrogen ion concentration in living tissues. Throughout his work Rous showed rare technical ingenuity, perhaps the most famous instance being his isolation of Kupffer cells in pure culture by loading them with particulate iron and collecting them from liver perfusates with the aid of a magnet. Another instance ahead of its time was his plating method for individual cells.

Rous's wholehearted and enthusiastic return to the cancer field was prompted by two personal episodes. A few years before, W. E. Gye had made certain claims as to the constitution of the Rous agent. Although these claims were not confirmed, their investigation had the undoubted effect of bringing the filterable sarcomata into a new prominence, and through this development Rous was both encouraged and stimulated—something for which he always expressed much gratitude. Secondly, the late Richard E. Shope had persuaded Rous to embark on a full-scale investigation of the so-called Shope rabbit papilloma virus recently discovered in the cottontail rabbit. Hence it came that Rous entered a quite new and highly productive phase which endured to the end of his life. Of special and lasting value were his studies of the biological interactions between chemical carcinogens and neoplastic viruses, and the generation of highly malignant carcinomata in the rabbit on the basis of the viral papillomas. In part, this experimental work led to, and was intertwined with, a fundamental conception (with Friedewald) which ranks, in the view of many, as perhaps the most profound of Rous's contributions; namely, the dissection of the carcinogenic process into two main stages of initiation and promotion. Independently advocated by Berenblum, the hypothesis remains influential and may still receive its explanation in chemical and cytological terms.

Dr Peyton Rous

At the time of the discovery of the avian tumour viruses, and for several decades thereafter, technical resources were inadequate to penetrate their structure and biochemistry. Although much remains to be done, great advances are in prospect which might dramatically or even decisively illumine the whole of the cancer problem. Meantime, in his later years Rous experienced great excitement from many discoveries on the part of others demonstrating the unexpected quality of the Rous virus, enhanced or in association with other agents, to break the species barrier and to evoke tumours in many species, both avian and mammalian; and secondly, from the immense resurgence of tumour virology to which his lone observation of 1910 had pointed the way.

Aside from his own publications (some 300 in all) Rous also contributed to scientific presentation and acted as editor of the *Journal of Experimental Medicine* for over thirty years. His width of cultivation could also be seen in most amusing and lighthearted essays, as on tactlessness, and the lamentable decline in self-satisfaction. Honours descended upon him from every part of the new world and the old, culminating in the Nobel Prize for Medicine (jointly) in 1966. For many reasons of domesticity and friendship he had special ties of affection for England and, with much other recognition, treasured his Linacre lectureship at Cambridge, his honorary fellowship of Trinity Hall, the Walker Prize of the Royal College of Surgeons, and his foreign membership of the Royal Society. In admiration he would refer to "those wonderful powder-blue volumes" (the Scientific Reports) of the Imperial Cancer Research Fund.

Rous was a true worker at the bench, yet believed that the experimental pathologist and the artist have much in common. He described his recreation as "relishing life", and this he did to the full. When the history of cancer research comes to be written, he will rank with the highest in honour.

Correspondence

Non-ideal Gas

SIR,—In view of the continuing flurry of polemics over the rights and wrongs of CS "gas", it is absolutely vital that the slender body of available factual evidence relating to the toxicity of the material be described with dispassionate accuracy. In the circumstances we have no alternative but to apologize and to assume full responsibility for a premature conclusion in our work on the toxicity of CS, which was reported in your excellent article[1]. Investigations carried out since these preliminary results were presented at the BSSRS symposium last November have revealed that the cytotoxic effects of injected CS on kidney and liver cells in mice are by no means clear-cut, and also that the primary locus of short-term toxicity resides in neither of these organs. In any case, this histological work is likely to prove irrelevant to the use of CS in riot control.

Some studies of CS toxicity have been published. Punte and his associates[2] at Edgewood Arsenal, Maryland, derived an approximate value for the LD$_{50}$ of 8 mg/kg body weight by intravenous injection into rabbits. These authors also showed that inhalation of CS aerosols by pigeons and by various mammals could cause death, and included LCt$_{50}$ values. Pulmonary oedema and haemorrhage of the adrenal glands were sometimes found, especially at high inhalation doses. Particles of CS also produced necrosis at sites of impact in both the gastrointestinal and respiratory tracts. As far as can be gathered, all experimental animals which have been subjected to CS aerosols were protected externally by hair or feathers. The comparison of such findings with the analogous situation in man is not therefore straightforward. Other studies have been carried out at Edgewood Arsenal and elsewhere, but, without denying the importance of making such studies public, the deduction that failure to publish automatically implies the discovery of disturbing features on the part of the material is a little facile.

When CS is employed in riot control, two things may happen. First, an amount of the agent may enter the body, and second, the subject may suffer cellular injury, most probably in the respiratory tract. If the Himsworth Committee confirms its original intention to consider CS as a drug, then the quantitative relationships between dose, effectiveness of action and toxic hazard become the crucial issue. The degree of exposure of any individual in a crowd can bear no resemblance to the defined dosage of a drug taken by a patient under the care of his physician. Moreover, very wide variation in the susceptibility of people to CS has been reported[3,4]. With knowledge of the action of poisons increasing rapidly and with techniques for detecting cytotoxicity becoming more sophisticated, we may soon find that any anti-personnel chemical causes some tissue damage somewhere, and hence constitutes a hazard to health. If experimental support for this concept is forthcoming, a humane society may have no alternative but to abandon the use of these materials entirely.

Yours faithfully,

G. R. N. JONES
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London WC2.

Syn and Anti

SIR,—In discussing the molecular conformation of nucleosides (*Nature*, **225**, 1190; 1970), your Biomolecular Structure Correspondent states that the pucker of the sugar ring in 4-thiouridine (Saenger and Scheit, *Angew. Chemie*, **8**, 139; 1969) is C2′ endo whereas the original paper states that the pucker is C3′ endo. In the case of pyrimidine nucleosides with a C3′ endo pucker the energy minimum for a *syn* conformation is not as low as that for the *anti* conformation, which makes the 4-thiouridine structure perhaps even more interesting than your correspondent suggests.

Yours faithfully,

HERBERT R. WILSON

Carnegie Laboratory of Physics,
University of Dundee,
Dundee.

Our Biomolecular Structure Correspondent writes: A typical C2′ endo sugar has C2′ $\sim$0·5 Å out of the plane defined by C1′, O1′ and O4′, on the same side as C5′, and C3′ $\sim$0·1 Å out of plane on the other side. In a C3′ endo sugar the out of plane displacements of C2′ and C3′ are reversed. In 4-thiouridine both C2′ and C3′ are displaced on the same side of the plane as C5′ by 0·3 Å and 0·8 Å respectively, and this rather unusual conformation might be described as "C2′ endo, C3′ endo". However, from the point of view of the orientation about the sugar-base link, the position of C2′ and its attached hydrogen is of particular importance and it was in this context that I described the conformation as approximating to C2′ endo. It may be that this description left an impression over-generous to the computerized seekers after energy minima; nevertheless, theirs is never the most rewarding of pursuits and I feel the 4-thiouridine observation provides them with some modest crumb of comfort which it would be churlish to ignore.

Butterfly Blues

SIR,—In Peter Scott's impressive catalogue (*Nature*, **225**, 1174; 1970) of the conservation projects of the World Wildlife Fund, one small inaccuracy needs correcting. The Large Blue butterfly, *Maculinea arion* Linn., should not be described as a species "threatened with world extinction". I can testify to its continued prevalence over much of the Swiss Alps and the Jura, and five years ago I recall finding it equally common in the foothills of the Caucasus at Sochi.

This makes it none the less desirable to save *M. arion*, if possible, from extinction in Britain. And I would like to stress the special value of *comparative* studies of the environmental pressures on such species, in areas where they are disappearing and in others where they continue to flourish. The need for this type of study is liable to be neglected—possibly because, involving prolonged field work by identical methods in more than one country, it may frequently demand more time and money than the amateur can give it. I hope the World Wildlife Fund will step in to support such an approach where it is required.

Yours faithfully,

C. GARRETT-JONES

c/o World Health Organization,
1211 Geneva 27,
Switzerland.

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[1] *Nature*, **226**, 95 (1970).

[2] Punte, C. L., Weimer, J. T., Ballard, T. A., and Wilding, J. L., *Toxic. Appl. Pharmac.*, **4**, 656 (1962).

[3] Owens, E. J., and Punte, C. L., *Amer. Ind. Hyg. Ass. J.*, **24**, 262 (1963).

[4] Punte, C. L., Owens, E. J., and Gutentag, P. J., *Arch. Envir. Health*, **6**, 366 (1963).

Antifertility Intermediate

SIR,—Male antifertility with 2,3-epoxypropanol was disclosed in two letters (*Nature*, **226**, 86 and 87; 1970). Jackson *et al.* suggest glycidol as an active intermediate of α-chlorohydrin. We propose an opposite mechanism—that glycidol is partially converted to α-chlorohydrin; both hypotheses consider the greater chemical reactivity of glycidol. Until these letters, no non-chlorinated related substances, including other halogens, were shown to be effective[1]. It seems more probable that glycidol would react with hydrochloric acid in the stomach to form α-chlorohydrin, glycerol and other substances—which explains the higher amounts required for activity. Hahn in his letter assumes the oxygen bridge of the active epichlorohydrin opens, in the rat, to form α-chlorohydrin, whereas opening the epoxy bridge of the active glycidol would yield inactive glycerol. The reactive glycidol, as we propose, opens up to form α-chlorohydrin in addition to glycerol.

The compound at the site of antifertility activity may indeed be α-chlorohydrin. Although 25–32 per cent of the compound is given off as $^{14}CO_2$ (labelled at the middle carbon) another 16–26 per cent is excreted in urine as the parent compound[2].

Yours faithfully,

R. J. ERICSSON
G. A. YOUNGDALE

Fertility Research,
The Upjohn Company,
Kalamazoo, Michigan 49001.

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[1] Ericsson, R. J., and Youngdale, G. A., *J. Reprod. Fertil.*, **21**, 263 (1970).

[2] Kirton, K. T., Ericsson, R. J., Miller, W. L., Cornette, J. C., Forbes, A. D., and Duncan, G. W., *Fed. Proc.*, **28**, 705 (1969).

Announcements

University News

Dr George Chadwick has been appointed to the second chair of town and country planning in the **University of Newcastle upon Tyne.**

Dr Ralph Riley, Cambridge Plant Breeding Institute, has been appointed a special professor in the Department of Botany, **University of Nottingham.**

Dr D. J. Greenland, University of Adelaide, has been appointed head of the Department of Soil Science, **University of Reading,** in succession to **Professor E. Walter Russell.**

Appointments

Mr R. H. Weir, director of the National Gas Turbine Establishment, has been appointed director of the **National Engineering Laboratory** in succession to **Mr F. D. Penny. Mr I. M. Davidson,** deputy director of the National Gas Turbine Establishment, has been appointed director.

In a series of appointments at the **Oak Ridge National Laboratory, Dr Floyd L. Culler** has been made deputy director from the middle of May—he will succeed **Dr H. G. MacPherson,** who is to become professor of nuclear engineering at the University of Tennessee. Four associate directors have also been appointed and are: **Dr James L. Liverman** (biological and environmental sciences); **Dr Arthur H. Snell** (basic physical sciences) who has been at the Oak Ridge National Laboratory since its beginning in 1958 and who has been responsible as an assistant director for work on controlled nuclear fusion; **Dr Donald B. Trauger** (reactor engineering sciences) who has been since 1964 director of the laboratory's advanced gas cooled reactor programme; and **Dr Frank R. Bruce** (administration). The laboratory has also set up a new Ecological Sciences Division under the direction of **Dr Stanley I. Auerbach.**

Miscellaneous

Dr Charles Stark Draper, the founder of the Instrumentation Laboratory (now the Draper Laboratory) at MIT, has been awarded the fifth Founders Medal of the National Academy of Engineering.

P. P. Feofilov has been awarded the C. I. Vavilov gold medal of the USSR Academy of Sciences for his work on spectroscopy and luminescence of activated crystals.

The Meldola Medal for 1969 has been awarded to **Dr P. W. Atkins,** Oxford, in recognition of his work in theoretical chemistry.

The Organisation Internationale de Lutte Biologique (OILB) is to be enlarged and restructured to form the new International Organization for Biological Control, to promote research into biological control in the broadest sense throughout the world. Provision will be made in the new statutes for individual, institutional and supporting membership, and for the creation of largely autonomous regional sections on a biogeographical basis.

ERRATUM. In the article "New Finds at the Swartkrans Australopithecine Site" by C. K. Brain (*Nature*, **225**, 1112; 1970), a penultimate sentence should be added to the paragraph beginning "In 1958 . . . " on page 1113: "A concentration of coarse material is visible in the outer cave filling, against the N. wall, just east of the face worked by Broom and Robinson".

ERRATUM. In the article "A Comparison of Marijuana Users and Non-users" by Norman E. Zinberg and Andrew T. Weil (*Nature*, **226**, 119; 1970), the third sentence in the first column on page 122 should be "There were no signs of overt intellectual deterioration in the C interviewees, . . .".

ERRATUM. The title of the communication by Mepham and Lane (*Nature*, **226**, 180; 1970) was given incorrectly on the contents page. It should read: "Adaptive significance of the sculptured exine of pollen".

ERRATUM. The book *Matrix Algebra for Business and Economics* was incorrectly priced at 40*s* (*Nature*, **225**, xxxii; 1970). It costs 140*s*.

International Meetings

May 19–21, **Non-ferrous Metals: the Next Fifty Years,** London (The Secretary, the British Non-Ferrous Metals Research Association, Euston Street, London NW1).

June 9–12, **Thirteenth Annual Meeting of the Canadian Federation of Biological Societies,** Montreal (Dr K. K. Carroll, Biochemistry Department, University of Western Ontario, London 72, Canada).

June 22–July 31, **Advanced Study in Gerontology** (summer institute), Los Angeles (Summer Institute Coordinator, Gerontology Center, University of Southern California, University Park, Los Angeles, California 90007, USA).

July 6–31, **Community Mental Health and Aging** (summer institute), Los Angeles (Summer Institute Coordinator, Gerontology Center, University of Southern California, University Park, Los Angeles, California 90007, USA).

September 1–4, **Hydraulic Transport of Solids in Pipes**, Coventry (Mr H. S. Stephens, Organizing Committee Secretary, 1st ICHTSP, British Hydromechanics Research Association, Cranfield, Bedford).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Furnished, 3-bedroomed, centrally heated house or flat for American physician and family, August 1970–71. Close to Mill Hill and within half hour from central London. Rent £50–£70 month. Please contact Dr A. Haase, Building 10, Room 11N-218, Bethesda, Maryland 20014, USA.

Wanted: British medical scientist and family require 2-bedroomed flat near Rockefeller University, New York, for 1 or 2 years from October 1970. Can exchange 3-bedroomed furnished house with garage and central heating in Ruislip Gardens (West London). Very conveniently situated for public transport. Please contact Dr T. J. Peters, Department of Medicine, Royal Postgraduate Medical School, Ducane Road, London W12.

Wanted : Furnished, centrally heated house or flat in or near northern London, for research social scientist and family (two children). Prefer one year lease starting between mid-June and mid-July 1970. Please contact Dr R. Turner, Department of Anthropology, University of British Columbia, Vancouver, BC, Canada.

Wanted : From August/September 1970, 3/4 bedroomed house in Bristol: convenient for university and Langford. Please contact Dr D. F. Kelly, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.

Wanted : 2–3 bedroom furnished house, Cambridge, 1 year beginning August 1, 1970, for professor and family. Prefer central heating; will consider 15 guineas p.w. Please contact M. Freedman, Anthropology Department, Syracuse University, Syracuse, New York 13210, USA.

Wanted : Furnished house or flat in or near Cambridge, England, for professor, wife and 2 children from September 1970 to September 1971. Central heating and 3 bedrooms preferred. **Vacant :** Can offer for rent or exchange 3-bedroomed house in Seattle, Washington, for same period. Please contact Dr Peter Hobbs, 2815, 105th Ave, SE, Bellevue, Washington 98004, USA.

Vacant : In central London, furnished 3-bedroom house, library, sitting room, dining room, well-equipped kitchen, full bathroom, second toilet, lovely small garden. Excellent bus and tube connexions to all areas. Available from late summer 1970, preferably for periods of more than six months. Please contact R. B. Fisher, 26 St Paul's Rd, London N1.

British Diary

Monday, April 27

Apprenticeship and Industrial Training (6 p.m.) Mr Cyril R. English, Royal Society of Arts, at John Adam Street, London WC2. (Second of three Cantor Lectures on Training for Industry.)

Contact Problems in Machines using Thyristor Assisted Communication (5.30 p.m.) Professor J. J. Bates, Mr S. Stanway and Mr R. F. Sansum, Institution of Electrical Engineers, at Savoy Place, London WC2.

Escalators and Travolators (6 p.m.) Mr C. W. Hardie, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Gunn Domain Logic (5.30 p.m.) Dr H. L. Hartnagel, Institution of Electrical Engineers, at Savoy Place, London WC2.

Quasi-stellar Objects (5.30 p.m.) Professor G. Burbidge (University of California), University College London, in the Physics Theatre, Gower Street, London WC1.

The Evolution of a Microbial Metabolic Pathway (5.30 p.m.) Professor Roger Y. Stanier (University of California), University College London, in the Botany Theatre, Gower Street, London WC1.

Tuesday, April 28

Biological Effects of Pollution in the Sea (two-day meeting) Royal Society at 6 Carlton House Terrace, London SW1.

Capacitors (10 a.m. symposium) Institution of Electronic and Radio Engineers, Components and Circuits Group, at 9 Bedford Square, London WC1.

Modulated Sub-carrier System for Attenuation Measurement at X-band (5.30 p.m.) Mr F. Warner, Institution of Electrical Engineers, at Savoy Place, London WC2.

Recent Developments in Tropical Agriculture (10 a.m. symposium), Society of Chemical Industry, Agriculture and Pesticides Groups, at 14 Belgrave Square, London SW1.

Selection of Materials for Marine Structures (6 p.m.) Mr H. Gibbs, Society of Chemical Industry, Plastics and Polymer Group, at 14 Belgrave Square, London SW1.

The Unicellular Blue-Green Algae—a Little Known Group of Bacteria (5.30 p.m.) Professor Roger Y. Stanier, University College London, in the Botany Theatre, Gower Street, London WC1.

24-Channel PCM (6.30 p.m.) Mr A. Stevens, Institution of Electronic and Radio Engineers, jointly with the Institution of Electrical Engineers, at the Civic Centre, Duke Street, Chelmsford.

Unsaturated C18 Acids—Their Synthesis and Properties (6.15 p.m.) Dr F. D. Gunstone, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Vehicle Handling Qualities, Professor F. D. Hales; **Measurement of Vehicle Handling by Tethered Testing,** Mr N. F. Barter (6 p.m.) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Wednesday, April 29

Electrical Power Installations in Warships (5.30 p.m.) Mr H. C. Fitzer, Institution of Electrical Engineers, at Savoy Place, London WC2.

Ionospheric Research by Means of Oblique Incidence Sounders (5.30 p.m.) Mr P. Bradley, Institution of Electrical Engineers, at Savoy Place, London WC2.

Marketing within the Plastics Industry (5 p.m.) Plastics Institute, at the Great Western Royal Hotel, Paddington.

Non-standard Analysis—II. Compactness Arguments in Function Theory (5.30 p.m.) Professor Abraham Robinson, University College London, in the Engineering Theatre, Gower Street, London WC1.

Quasi-stellar Objects—II (5.30 p.m.) Professor C. Burbidge, University College London, in the Physics Theatre, Gower Street, London WC1. (Further lecture on April 30.)

Scanning Circuits for 100° Colour Tubes (6 p.m.) Mr K. E. Martin, Institution of Electronic and Radio Engineers, Communications Group, at 9 Bedford Square, London WC1.

The Origin of the Earth's Magnetic and Gravitational Fields (5 p.m.) Dr R. Hide, Royal Meteorological Society, at 49 Cromwell Road, London SW7. (Symons Memorial Lecture.)

Thursday, April 30

Cooperation between Lymphocytes in the Immune Response (2.30 p.m. discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Microprogramming and Processor Design (5.30 p.m.) Professor M. V. Wilkes, Institution of Electronic and Radio Engineers, Joint IEE/IERE Computer Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

The Hydrogenation of Fatty Acids (6.30 p.m.) Dr Brangwin, Institution of Chemical Engineers, at Whitehaven College of Further Education, Whitehaven.

Friday, May 1

Logic as the Science of Mathematical Reasoning—III (5.30 p.m.) Professor Abraham Robinson, University College London, in the Engineering Theatre, Gower Street, London WC1.

Spectroscopic Properties of Aromatic Chromophores in Surfactant Systems (1 p.m.) Dr J. Philp, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

The Invisible Man (9 p.m.) Professor E. M. Rogers (Princeton), Royal Institution, at 21 Albemarle Street, London W1.

Monday, May 4

Assessing Markets for Entirely New Products (6 p.m.) Mr H. M. Kelner Society of Chemical Industry, at 14 Belgrave Square, London SW1.

London Engineering Congress (four days) Council of Engineering Institutions, at the Royal Festival Hall, London SE1. Theme: The Challenge Facing the Profession in its Services to the Community.

Vacuum Arcs (5.30 p.m. discussion meeting) Institution of Electrical Engineers, at Savoy Place, London WC2.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Building Research Station. Current Paper 48/69: Application of Scanning Electron Microscopy to Building Materials. By A. J. Majumdar, K. Speakman, K. W. J. Treadaway and R. C. De Vekey. Pp. 9 + 33 figures. (Garston, Watford: Building Research Station, 1969.) *Gratis.* [102

Proceedings of the Royal Irish Academy. Vol. 69, Section A, No. 1: Mechanisms of Electron Loss in a Hydrogen Afterglow. By J. C. Cronin and M. C. Sexton. Pp. 1–10. *2s 6d.* Vol. 69, Section A, No. 2: Equations of Motion in General Relativity. By J. L. Synge. Pp. 11–38. *7s.* Vol. 69, Section B, No. 1: Petrology of the Metamorphosed Pre-Cambrian Rocks of South-Easternmost Co. Wexford. By J. W. Baker. Pp. 1–20 + plates 1 and 2. *5s.* Vol. 69, Section B, No. 2: Antitubercular Substances—XXI. Synthesis of 2-Nitrodiphenylamines. By J. G. Belton and Mary McInerney. Pp. 21–30. *2s.* (Dublin: Royal Irish Academy, 1970.) [112

Water Resources Board. The Ground-Water Hydrology of the Lincolnshire Limestone with special reference to the Ground-Water Resources. By R. A. Downing and B. P. J. Williams. Pp. xiv + 160 + 4 plates. (Reading: Water Resources Board, 1969.) *140s.* [112

The Natural Rubber Producers' Research Association. Index of Publications, 1938–1969. Pp. 116. (Welwyn Garden City: The Natural Rubber Producers' Research Association, 1970.) [122

Symbols, Signs, and Abbreviations recommended for British Scientific Publications. (A Report by the Symbols Committee of the Royal Society, representing The Royal Society; The Chemical Society; The Faraday Society; The Institute of Physics and the Physical Society.) Pp. 44. (London: The Royal Society, 1969.) *7s.* [122

Transistor–Transistor Logic 9000 Series—Applications Handbook. Pp. 70. (Witham, Essex: Marconi-Elliott Microelectronics, Ltd., 1970.) *30s.* [122

Pesticide Science, Vol. 1, No. 1 (January–February 1970). Pp. 1–44. Subscription: Members *80s*; Non-members *160s*. Single issues *27s 6d.* (London: Society of Chemical Industry, 1970.) [122

Building Research Station. Current Paper 47/69: Examples from Bills of Quantities (Operational Format). By E. R. Skoyles. Pp. 6. (Reprinted from The Quantity Surveyor, 1969, Vol. 25 (6), May and June.) (Garston, Watford: Building Research Station, 1969.) *Gratis.* [132

The Zoological Record, Vol. 104, Section 10, 1967: Crustacea. Part 1 (Recent) compiled by Anandi Balasubramanian and Patrick D. Armitage. Part 2 (Fossil) compiled by Carolyn Roberts. Pp. vi + 160. (London: The Zoological Society of London, 1970.) *40s.* [162

Field Studies Council. Annual Report 1968–69. Pp. 55 + 8 plates. (London: Field Studies Council, 1970.) [162

The Role of Public Research and Development in Exploiting Technology. By John Lyons. (Paper presented to a seminar of the Science of Science Foundation in London, 3 December 1969.) Pp. 25. (London: Institution of Professional Civil Servants, 1970.) [162

Annual Report of the National Museum of Wales, 1968/1969. Pp. 102. (Cardiff: National Museum of Wales, 1969.) [162

The Physics Exhibition Handbook 1970. Pp. 294 + 40. (London: The Institute of Physics and the Physical Society, 1970.) [162

Annual Report of the Leicester Museums for the period 1st April, 1967–31st December 1968. (62nd Report to the City Council.) Pp. 82. (Leicester: Leicester Museums, 1970.) [162

The International Journal of Biochemistry, Vol. 1, No. 1 (February 1970). Pp. 1–128. Edited by G. A. Kerkut, assisted by Robert J. Walker. Annual subscription *300s*; $40. Single copies *60s*; $8. Published bimonthly. (Bath: Scientechnica (Publishers), Ltd., 1970.) [162

Other Countries

US Department of the Interior: Geological Survey. Bulletin 1258-D: Stability of the West Slope of Government Hill Port Area of Anchorage, Alaska. By David J. Varnes. Pp. v + 61 + 3 plates. Professional Paper 496-B: Geochemistry and Paleolimnology of the Trona deposits and Associated Authigenic Minerals of the Green River Formation of Wyoming. By W. H. Bradley and H. P. Eugster. Pp. v + 71 + 2 plates. Professional Paper 679: Geology, Petroleum Development, and Seismicity of the Santa Barbara Channel Region, California. By J. G. Vedder *et al.* Pp. ix + 77 + 3 plates. [211

Records of the Dominion Museum, Wellington. Vol. 6, No. 14: Classification of New Zealand Arionacea (Mollusca: Pulmonata). 2: A Revision of *Charopa* Subgenus *Ptychodon* Ancey, 1888. By F. M. Climo. Pp. 175–258. Vol. 6, No. 15: Classification of New Zealand Arionacea (Mollusca: Pulmonata). The Systematic Status of *Phenacohelix* SP. Suter 1909. By F. M. Climo. Pp. 259–262. Vol. 6, No. 16: Two New Heart-Urchins, including a New Species of *Cyclaster* from New Zealand Waters (Echinoidea: Spatangoida). By Alan N. Baker. Pp. 265–273. (Wellington, NZ: Dominion Museum, 1969.) [221

World Health Organization. Technical Report Series, No. 434: African Trypanosomiasis—Report of a Joint FAO/WHO Expert Committee. Pp. 79. (Geneva: World Health Organization; London: HM Stationery Office, 1969.) 4 Sw. francs; *8s*; $1.25. [221

The Harvey Monroe Hall Natural Area. By Jens Clausen. Pp. 48. (Stanford, California: Carnegie Institution of Washington, Department of Plant Biology, 1969.) [221

Insulin in Serum: a Review. By Flemming Gjedde. Pp. 61. (Supplement 7 to Vol. 16, 1969, of *Danish Medical Bulletin.*) (Copenhagen: Costers Bogtrykkeri, 1969.) [221

International Atomic Energy Agency. IAEA Science Features, No. 1, January 1970. Pp. 16. (Vienna: IAEA, 1970.) [231

Health Aspects of Chemical and Biological Weapons—Report of a WHO Group of Consultants. Pp. 132. (Geneva: World Health Organization; London: HM Stationery Office, 1970.) 12 Sw. francs; *24s*; $4. [261

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 38, Fasc. 7: Principes de Phylogénèse Animale Appliqués à l'Interprétation des Métazoaires Diploblastiques et à leur Evolution en Métazoaires Triploblastiques. Par Paul Brien. Pp. 110. (Bruxelles: Academie Royale de Belgique, 1969.) 140 francs. [261

Field Museum of Natural History. Fieldiana—Zoology. Vol. 51, No. 11: The Larva of the Frog *Leptodactylus hylaedactylus* (Leptodactylidae.) By W. Ronald Heyer and Philip A. Silverstone. Pp. 141–146. Vol. 51, No. 12: Veronicellid Land Slugs from the New Hebrides, with Description of *Semperula solemi*, New Species. By Lothar Forcart. Pp. 147–156. (Chicago: Field Museum of Natural History, 1969.) [271

Tropical Fish Culture Research Institute. Report for 1968. Pp. 30. (Malacca, Malaysia: Tropical Fish Culture Research Institute, 1969.) [271

Indian Council of Medical Research. Annual Report of the National Institute of Nutrition for the period 1st October 1968 to 30th September 1969. Pp. viii + 118. (Hyderabad: National Institute of Nutrition, 1969.) [271

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Animal Physiology, 1968/1969. Pp. 75. (Sydney: CSIRO, 1969.) [281

Annual Report for the Durban Museum and Art Gallery for the municipal year 1968/1969. Pp. 9 + 4 plates. (Durban: Museum and Art Gallery, 1969.) [281

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Building Research 1968/1969. Pp. 91. (Melbourne: CSIRO, 1969.) [281

Science Council of Canada. Special Study No. 8: Scientific and Technical Research in Canada, Part 2. Chapter 1: Government Departments and Agencies. Pp. xi + 168. (Ottawa: Queen's Printer, 1969.) $1.75. [281

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 138, No. 6: Lizards and Snakes from Southwestern Asia, Collected by Henry Field. By Georg Haas and Yehudah L. Werner. Pp. 327–406. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1969.) [291

Astronomy and Astrophysics Supplement Series, Vol. 1, No. 1 (January 1970). Pp. 1–114. (Leiden: The Observatory, 1970.) [291

Bulletin of the American Museum of Natural History. Vol. 142, Article 2: Function of Forebrain and Cerebellum in Learning in the Teleost *Tilapia heudelotii macrocephala*. By Harriet Kaplan and Lester R. Aronson. Pp. 141–208. (New York: American Museum of Natural History, 1969.) $2.05. [291

Studies in Colour Vision. By Georg H. M. Waaler. Pp. 32. (Oslo: Universitetsforlaget, 1969.) Nkr. 12. [301

Inorganic Macromolecules Reviews, Vol. 1, No. 1 (January 1970). Published quarterly. Edited by F. G. R. Gimblett and K. A. Hodd. Pp. 1–88. Subscription price: Dfl. 90 plus Dfl. 4.50 postage (approx $25 plus $1.25). (Amsterdam: Elsevier Publishing Co., 1970.) [301

Smithsonian Contributions to Zoology. No. 22: A Revision of the Melanesian Wasps of the Genus *Cerceris latereille* (Hymenoptera: Sphecidae). By Karl V. Krombein. Pp. 36. $0.45. No. 25: A Monographic Study of the Mexican Species of Enlinia (Diptera: Dolichopodidae). By Harold Robinson. Pp. 62. $0.65. (Washington: Smithsonian Institution Press, 1969. Available from US Government Printing Office.) [301

US Department of the Interior: Geological Survey. Professional Paper 603: Uranium Resources of Northwestern New Mexico. By Lowell S. Hilpert. Pp. v + 166 + plates 1–4. (Washington, DC: Government Printing Office, 1969.) [301

US Department of Health, Education and Welfare: Public Health Service. Down's Syndrome (Mongolism)—a Reference Bibliography. By Rudolf F. Vollman. Pp. iv + 88. (Washington, DC: Government Printing Office, 1969.) $0.45. [22

European Organization for Nuclear Research, CERN. Proceedings of the Neutrino Meeting, CERN, Geneva, 13–14 January, 1969. Edited by J. B. M. Pattison, C. A. Raum and W. A. Venus. Pp. 170. (Geneva: CERN, 1969.) [22

Annals of the New York Academy of Sciences. Vol. 167, Article 1: Comparative and Evolutionary Aspects of the Vertebrate Central Nervous System. By J. M. Petras, C. R. Noback and 35 other authors. Pp. 1–513. (New York: New York Academy of Sciences, 1969.) $29.50. [32

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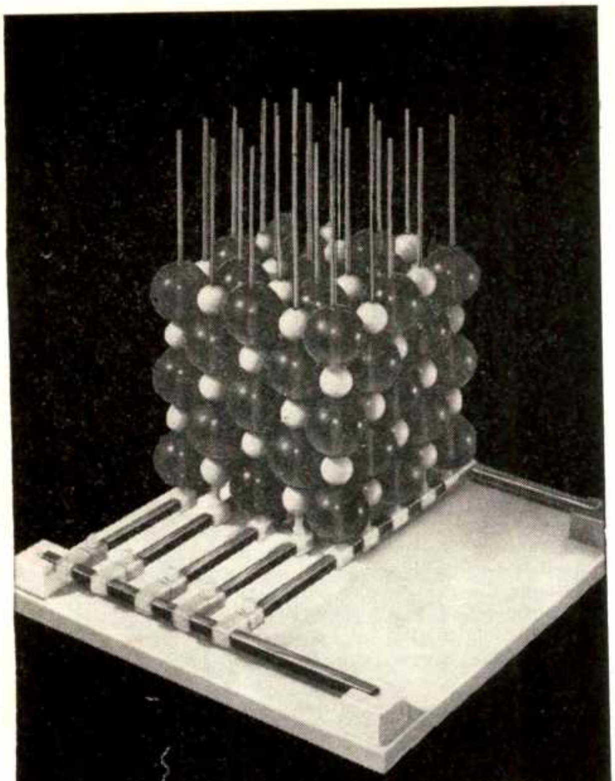

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(1557)

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(1493)

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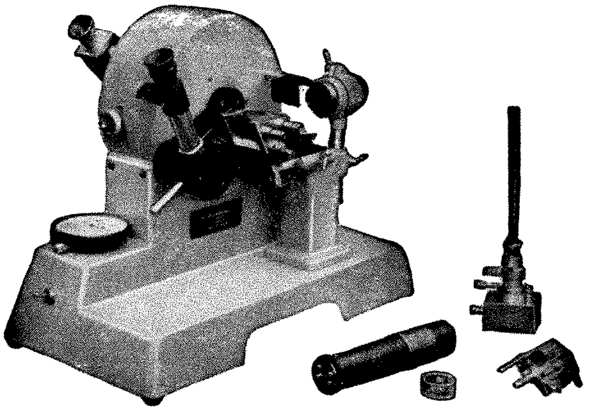

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NATURE Volume 226
MAY 16, 1970

What Future for American Universities?

THE past few weeks have caused many sober people to question the continued viability of universities in the United States. The shooting of four students at Kent State University, Ohio, two weeks ago is only one of the more dramatic signs of crisis. For the past two years, the cross currents within the academic system which have progressively complicated and impeded the process of teaching have been plain for all to see. It has nevertheless been a great surprise to see how trouble on the campus has spread from places such as Berkeley to universities such as Yale which quite recently were sneered at by the radicals for their primness and to universities such as Kent State, which until recently were remarkable only for their size. But student protests are only one symptom of underlying malaise. Over the years, and particularly this year, the extent to which scholars are supported in their research by the Federal Government has declined, so that there is a bleak prospect that the newer institutions in the United States will never graduate to the first division of academic institutions. Then there is confusion about the fitness of the curriculum for the modern world, and it has rarely helped as much as some academics hoped that several universities have bent their principles to accommodate the demands of students for instruction in this or that. But the universities are also under fierce economic pressure, and the past few weeks have been filled with announcements of higher fees for tuition in the coming academic year and with the gloomy prospect that several worthy institutions will in future be compelled to economize in the quality of what they do for students.

Where will all this lead? Although the pressures with which the university system is now confronted could probably be traced by a sufficiently energetic sociologist to some common cause, possibly a permanent change in the character of American society, universities must live from day to day and there is probably some benefit in seeking to disentangle the threads of the present crisis. The increasing involvement of the student body at many universities, and the faculty as well at many of them, has been in many ways the biggest surprise. A decade ago, after all, American universities were outstanding for their hard-working dedication to the accumulation of academic credit and for their indifference to what was happening outside. To be sure, Dr Nathan Pusey, president of Harvard, was one of several people who stood up to Senator J. McCarthy in the middle fifties, which is why it is so ironic that he should have been, with some justice, the target for much of the invective against university administrations in the past few months. (Harvard is now in the throes of choosing a new president for itself, and it will be interesting to see whether the high hopes now entertained that some kind of messiah will emerge from the selection process turn sour when, almost inevitably, they are disappointed.) At the beginning, even the Vietnam war did not set the campuses alight. Social issues, especially the racial problems of the United States, seemed among the first to draw American students into political activity. Senator Eugene McCarthy's attempt to win the Democratic nomination in 1968 was a sign that organized students could have an external influence on political events. With the invasion of Cambodia, there is no prospect that they will be ready to overlook the opportunities which now exist.

That university people should be engaged with political events is in principle entirely proper. Indeed, there is something in the old complaint that universities in the English-speaking world have traditionally been too indifferent to what goes on about them. It is, however, essential that everybody concerned should recognize that it is bound to be a gross interference with academic and even personal liberty to attempt to create situations in which individual universities can speak with one voice. In other words, if a faculty meeting or a student union meeting wishes to pass political resolutions and draw these to public attention, well and good. But it is not feasible to ask that universities in their corporate incarnations should take political positions—that would imply the suppression of disagreement within the universities, which would itself be an intolerable misinterpretation of what a university is for. To be sure, a lot of clamour within a university on some public issue can give outsiders the impression that universities as such are committed to the same view, but this interpretation is mistaken and should be described as such by any academic whenever the chance arises. One of the least pleasing features of the recent wave of protest from students and academics is that there have been signs of attempts to persuade the reluctant to put their names to petitions in which they do not trust, or to lend their presence for causes they do not share. It follows from this that if some sections of the university are proposing to exercise their legitimate right to engage in external political activities, it is unavoidable that all who work in the universities will sooner or later be drawn into a more direct concern with political events, if only so that they know as individuals where they stand. In the long run, if handled wisely, this development could be beneficial. After all, informed debate on political issues within universities could help enormously to clarify the public mind. One of the defects of the universities in the past few months is that those who have no wish to join the picket lines have been too passive. The invasion of Cambodia may be indefensible, but it has not been in the public interest that the universities have provided a great many of the more hot-headed leaders for what they call the battle to preserve the environment without there being any measured protest within the universities that a good cause may be being spoilt by exaggeration.

So does it follow that the universities can influence political events only to the extent that individual academics and students may have time and energy for political activities, and to the extent that their disputes with each other may be educative for the world at large? Evidently there is more to be done than this. For one thing, academics are usually more able than other people to make their views heard—they have the words and often the time. Plainly Mr Kingman Brewster, the president of Yale, has had a marked influence with his declaration in April that black revolutionaries could not easily find a fair trial in the United States, and by his help with student protest at Yale on May 1 against the treatment of Black Panthers. Similarly, the students—possibly 100,000 of them—who congregated outside the White House in Washington last weekend have clearly forced the government to acknowledge that they have a case—conceivably, they would have been even more influential if they had avoided mixing up with the issue of Cambodia all kinds of other worthy but irrelevant causes—the Black Panthers and Women's Liberation, for example. To be sure, there are dangers. The rough handling of a group of student activists by building workers in New York last week shows that the fondness of university students for the cause of what they call the working class is not always reciprocated, and there is a serious danger that the rival of political interest and energy in the universities may run into the sand by being too isolated from reality. In the long run, that could be damaging for the institution of the university. No doubt the building workers in New York last week were fully aware that the taxes which they pay are necessary to keep the universities alive.

Externally, then, the universities should not be afraid of being dragged into politics but should find ways of allowing this to happen without damaging academic freedom or the reputation of the universities for good sense. Perhaps the most valuable model is that devised at Princeton, where the university now plans to close for two weeks before the election next November so that students and faculty can do more to influence local politics. Internally, too, universities need to be more courageous. In retrospect, the demand of students to be involved with the administration of American universities has in the past been acceded to unnecessarily. It is, of course, right that students should have more say in the conduct of their own lives, but it is not sensible that they should have a direct influence in the planning of the curriculum. To be sure, there is plenty of room in every university for arguing about what is taught and how, but it is hard to accept the good sense of the arrangements made in some universities for teaching subjects which are hardly academic disciplines in an independent sense. The departments of black studies in several American universities have become relics of the period a few years ago when this cause came at the top of the revolutionary list. Over the years, the universities have been grudging in their response to the legitimate demands of students and weaker than they should have been in their resistance to unreasonable demands. The truth is, of course, that there is a difference of principle between the interests of students and of faculty; teachers are paid to teach what they can teach and students elect to listen to them. None of this justifies complacency about the present curriculum, but it would be as well if the present mood towards more vigorous discussion of external issues were also accompanied by a determination, principally by the faculty, to strike the right balance within the administration of the universities as well.

The external pressures to which universities are subjected is another serious cause for anxiety. Everybody knows by now that the upheaval in the universities has not made conspicuous friends among the taxpayers. Congress is equally inclined to listen to jaundiced views. And in any case, there are huge structural problems in trying to match financial support for university education with the common and reasonable assumption that higher education is something which everybody deserves. Already there are alarming signs that universities which are dependent on state governments for their financial support are being looked over with uncomfortable care by the boards of regents, so that many academics fear that it cannot be long before appointments are determined in the state house. Certainly there has been a marked disinclination already for state governments to help with the financing of university research—an activity which in happier times was regarded as a feather in the cap by the regents of the University of California. A part of the trouble may be that the comparatively recent invention of bureaux of the budget by the state governments has not yet had time to settle down. More probably, however, parsimony and petty interference have come to stay.

This is why it is important that the sense of unity within universities which seems to have been engendered by the external crisis should be used not merely as a way of persuading the American army out of Cambodia but also as an incentive to find a common defence of the university system against the pressures which are undermining it, externally and internally alike. In the long run, some better way has to be found for letting students and faculty coexist. The problem is to let students feel free and to permit faculty members to remain scholars. Nobody should sneer if it turns out that the external crisis which has caused the students to go marching serves also as a spur for putting the university system in order.

From Gentleman to Player

WHATEVER future is decreed for the Family Planning Association by the negotiations now in progress with the Department of Health and Social Security, it is no mean triumph that the government has finally acknowledged an active responsibility in this area. Concern about the growth of Britain's population, which may in part have prompted this change of heart, is an entirely separate and on the whole less pressing issue than that of providing all who need it with genuine access to modern methods of contraception. At present that access is denied, as the abortion rates alone will testify. But this is not to detract from the major expansion that the Family Planning Association has undergone in the last five years. It has doubled the number of clinics and raised to more than half a million the number of people who seek advice from them each year. The expansion has, of course, been helped by the 1967 Family Planning Act which empowered, but did not compel, local authorities to provide contraceptive services to all who required them, regardless of marital status. But the Association has had an uphill task to take advantage of this long overdue legislative support—according to a recent survey, only a quarter of the local authorities are now providing the full services recommended by the act.

The recent expansion has hastened the transition of the Family Planning Association from voluntary to professional status. The days of the great pioneers and of wrestling with the prejudices of the medical profession are ending, though not yet over. But now that the battle for public support has been largely won, the Family Planning Association is divided as to what its next goal should be. It is natural that differences of opinion should arise between those who see the major task of the Association as being to dispense contraceptive advice to the maximum number of people and others, particularly the medical staff, who wish to give consultation on sexual problems to a smaller number of people.

These differences may have been responsible for the recent resignation of Dr Hilary Hill, the deputy director (medical), which seems to have hastened the Association's plans for reviewing the medical side of its activities. A commission under Lord Platt has now recommended that the Association appoint a chief medical officer who would be backed by a smaller and more active panel of advisers than the present medical council. These proposals, which have been accepted by the national executive committee of the Association, reflect the growing technicalities of contraceptive practice and the need for the Association to provide authoritative medical opinions on advances in reproductive technology as well as on occasions when government committees or newspapers provoke unnecessary despondency about the pill.

The side-effects of the pill that are now reaching statistical significance have produced several kinds of headache for family planning administrators. There is not just the flight from the pill that alarmist pronouncements engender. The proliferation of medical tests which doctors like to give before prescribing the pill may reassure some women but inhibits others, particularly those who are liable to be intimidated by the clinical procedures. Unfortunately it seems that the latter category includes the type of women who stand most in need of family planning advice. This is one of the questions that is to be studied by the major survey of family planning services that is to be mounted shortly by the Department of Health and Social Security. The future status of the Family Planning Association may well be affected by the negotiations now under way, but even if in the name of integration the Association disappears into the arms of the health service, there could be no more significant tribute to the way in which it has tackled responsibilities which governments have shirked for so long.

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100 Years Ago

The Royal Society

I CANNOT but think that the list of candidates recommended by the Council for election into the Royal Society published in your last number will be read by the outside world with considerable surprise. I look in vain in it for the names of two men, at least, of world-wide reputation, and well known as no mere *dilettanti* in their respective sciences, who were among the candidates, while the names of others are found there, which are on everybody's lips with the thought, What have they done to merit the scientific distinction which is looked on by every lover of science as almost an opening of the gates of paradise? Is it possible for us outsiders to learn anything of the considerations which govern the election? NOT AN F.R.S.

THE *British Medical Journal* states that the chair of Physiology, in the University of Prague, vacant by the death of the celebrated Purkinje, has been filled by the appointment of Dr. Hering, of Vienna. It was offered to Professor Helmholtz, who, however, preferred to remain at Heidelberg.

A RECENT number (94) of the German series known as "A Collection of Popular Scientific Treatises, edited by R. Virchow and Fr. von Holtzendorff," is a lecture on the Glacial Period (*Die Eiszeit der Erde*), by Alexander Braun. It gives a clear and concise history of the observations and arguments by which geologists have been led to the conclusion that a lengthened period of extreme cold overspread the greater part of Europe before the commencement of the historical epoch.

From Nature, 2, 48 and 51, May 19, 1870.

OLD WORLD

DEFENCE

Whittled Down Planes

THE decision to go ahead with the next step in the project to build what is known as the Multi-Role Combat Aircraft is now expected to be delayed for three or four weeks. The previous stage of project definition was completed at the end of February, and the agreement to begin the development stage, now involving the construction of seven prototypes to be followed by six preproduction aircraft, was to have been signed on May 1. But last month's announcement of a change in the requirements of West Germany, which is a partner in the project with Britain and Italy, appears to have reopened discussions of the basis on which the project is to be carried out. Meanwhile in Britain there is some unease about the project; the aircraft is the natural successor to the TSR-2 cancelled in 1965, memory of which still rankles. In particular there is a certain measure of public sensitivity about the series of cancellations of military aerospace projects which between 1951 and July 1967 are said to have cost more than £430 million. Recent remarks which the West German Minister of Defence, Mr Helmut Schmidt, is reported to have made in Bonn last week, which are thought to imply that the German requirements for the MRCA might be altered again, have not added to confidence in the project.

As the project stands at present the expectation is that Britain and West Germany will each take 400 of the aircraft, the design of which has not yet been made public, and Italy is to take 100. These figures represent a considerable whittling down of the original estimates which at one time had 600 aircraft on order for West Germany and 200 for Italy, although the British requirement seems to have risen slightly from 385 to 400. West Germany announced last month not only a reduction in its order but also a decision to take only the two-seat version instead of the mixture of single-seat and two-seat aircraft which were to make up the original 600.

Because of the alteration in the German position, the launching of the development phase has had to be postponed. The situation is complicated by uncertainty about the attitude of the junior partner, Italy, which after the German decision was the only member still wanting the single-seat version of the MRCA. The Italians have now also decided not to order the single-seat version, but their views on the two-seater are not known. It is considered possible but not likely that Italy may wish to drop out of the project altogether.

Confidence in the project has been further shaken by Mr Schmidt's recent pronouncement in which he apparently succeeded in implying that the German requirement for the MRCA may be reduced yet again. This was read into a statement that the ill-fated Starfighters of the West German Air Force are to continue in service longer than expected, to the end of the seventies, and that the German requirement for the MRCA would be 420 at most. The original plan was for the MRCA to replace the Starfighter in the German Air Force. Meanwhile in the House of Commons the British Minister of Defence, Mr Denis Healey, has been parrying questions about the cost of the project which, before the decision to proceed only with the two-seat version, was thought to be a total of about £470 million for the research and development work. On top of this is the unit cost for each aircraft, believed to be about £1·86 million for the two-seat version.

RESEARCH COUNCILS

Giving to Him who Hath

THE Science Research Council faces the permanent dilemma of whether to concentrate its support on a few centres of excellence or to spread its resources thinly among all the possible claimants, few of whom may achieve excellence in any field. The former strategy leads to charges of favouritism; the latter would threaten the quality of the national research effort. Moreover, the government expects that at least some of the £100 million entrusted yearly to the SRC will be devoted towards national goals, however ill-defined these may be.

What then is to be done? According to Sir Brian Flowers, chairman of the SRC, the best policy is to steer a middle course but at the same time to lean heavily towards a few institutions where expertise has been built up and where the resources will be used to their best advantage. Sir Brian, who was addressing the industrial committee of the Science of Science Foundation last week, lamented the way in which economic crises and mounting disillusionment with science have cut the rate of growth of science budgets to inadequate levels and forced an even greater measure of selectivity on the SRC.

About a quarter of the SRC's grant is spent in areas which the council wishes particularly to stimulate, such as astronomy and computers, while the rest goes on grants for individual projects. Applications for money should be judged by five criteria, Sir Brian said. Will the project contribute to the internal well-being of that particular subject? Will it help other branches of science? What are the economic, social and cultural implications of the project? It is inevitable that the largest slices of the SRC's cake go to institutions which have built up centres of excellence in particular fields so that in the course of time resources become more and more concentrated with the strong universities becoming stronger and the weak, in relative terms, weaker.

The Social Science Research Council does not have the same problems because the type of research it supports does not require large capital expenditures and there is no similar pressure to concentrate its resources. Mr Andrew Shonfield, the chairman of the SSRC, pointed out at the same meeting the problems in assessing the possible significance of a social science research project. Many of the social sciences are in a formative stage of their development and, according to Mr Shonfield, the best that can be expected from social science research in any case is that it "might be able to develop a few social indicators which tell us where we are now". It is therefore unrealistic to support social science research for its practical possibilities alone and the SSRC should be guided by other criteria as well. One of the council's aims is to encourage team work, especially interdisciplinary team work, but this policy runs up against the problem that most social science research workers wish to be heroes in their own right, and are less ready to collaborate than are natural scientists. There is also the problem, Mr Shonfield said, that some social scientists believe that interdisciplinary work taints them.

COMPUTER POLICY

ICL's Feather Bed

THE final public session this week of the investigation of the British computer industry which is being carried out by a subcommittee of the House of Commons Select Committee on Science and Technology has revealed the extent to which the major British manufacturer of hardware, International Computers Limited, is favoured by government orders. ICL received 99 per cent by value of central government orders for administration and general purpose computers placed in the year ending March 31, 1969. But the situation was less favourable for ICL during the last financial year, when its share of orders fell to 67 per cent.

These figures were given to the subcommittee in a memorandum from the Civil Service Department, which defines its role in the computer field as the central coordinator which ensures that government policy on the use of computers within departments is correctly applied. During the remainder of 1970, according to the memorandum, the government expects to order £20 million worth of computers of which ICL may supply up to 80 per cent.

Leading the team from the Civil Service Department and from the Stationery Office, which deals with the mechanics of the purchasing process, was the deputy secretary of the Civil Service Department, Mr N. G. Morrison, who gave the clearest definition of the rules applying to the choice of computers for government use that the subcommittee has heard. With two important exceptions, the process is to seek competitive tenders from three firms including one which offers equipment manufactured in Britain. The contract is awarded on merit, but there is a preference in favour of British machines provided there is no undue price differential. But Mr Morrison refused to specify in public session the size of the allowed price differential, alleged by Mr E. Nixon of IBM (UK) to be 25 per cent, although in an earlier session Sir John Wall, chairman of ICL, denied any knowledge of a preference favouring ICI.

The exceptions to the rule are computers more powerful than Atlas which are acquired from ICL without requesting tenders from other manufacturers, subject to the conditions of satisfactory price, performance and delivery dates. This applies to machines such as the ICL 1906A and System 4-70. Smaller computers are also acquired by requesting only one tender, usually from ICL, when they are intended to precede the use of larger machines from the same family or where there are other reasons for buying a particular machine such as compatibility, again subject to price, performance and delivery.

The policy of single tendering has of course been worrying the subcommittee and the extent to which ICL has benefited was given in the Civil Service Department memorandum (see Table). Mr Ted Leadbitter of the subcommittee, for example, wondered how the decision on single tenders could be subject to price, performance and delivery date when no other manufacturer had been consulted. But the witnesses thought that single tendering was the most efficient way of obtaining large systems.

CENTRAL GOVERNMENT ORDERS FOR COMPUTERS

	Value of all orders with all manufacturers £1,000	Value of all orders with ICL £1,000	Value of single tender orders with ICL £1,000
Orders placed (April 1, 1968, to March 31, 1969)			
Administration and general purpose	7,590	7,490	4,440
Scientific purposes	2,225	1,720	
Orders placed (April 1, 1969, to March 31, 1970)			
Administration and general purpose	13,025	8,780	8,350
Scientific purposes	4,260	325	75
Total	27,100	18,315	12,865

How the mechanism of the procurement of computers for use in government departments might be simplified from the present system, which apparently involves the Civil Service Department, the Stationery Office, the Treasury, and the Ministry of Technology in addition to the department which is receiving the computer, is also being discussed by the subcommittee. To the suggestion that the part played by the Stationery Office is taken over by the CSD, Mr Morrison said that the CSD has no experience of ordering and he doubted whether anything would be achieved by transferring staff from the Stationery Office to do the job. Mr Leadbitter thought that a lot of woolliness would vanish if the CSD and the Stationery Office were removed from the scene and the evaluation of tenders and procurement were done by the user department. But the witnesses felt it important to have a central coordinating body such as the Management Services Division of the CSD which deals with computer affairs.

The interrogation of Mr Morrison and his colleagues marked the end of the public sessions of the examination of the prospects for the British computer industry which has been occupying the subcommittee since February. Computer manufacturers, the software industry, large users of computers and the Ministry of Technology have given evidence to the subcommittee, which has become noted for its thoroughness. The subcommittee's report is expected to be available by July.

ASTRONOMY

Sic Transit Gloria Mercurii

FULL honours were done at the Royal Greenwich Observatory last Saturday to the transit of Mercury across the Sun's disk. On the lawn of the observatory a forest of antique telescopes was pointed defiantly through the blanket of clouds at the putative position of the Sun. An enthusiastic group of observers who had been manning their instruments since the beginning of the transit at 4 a.m. stood around like so many stout Cortezes, waiting for a break in the clouds and the planet to swim into their ken. The purpose of the occasion was to celebrate the transits of Venus and Mercury observed by Captain Cook 200 years ago, the former from Tahiti, which was the original purpose of his voyage to the southern hemisphere, and the latter from Mercury Bay, New Zealand. It was after this observation that he charted New Zealand and discovered the west coast of Australia.

The reason for sending Cook to observe the transits of Venus and Mercury from the southern hemisphere was to measure the planets' parallaxes, which at that time offered the best hope of calculating the mean distance between the Earth and Sun. The occasion was also notable for being the first really international scientific event—altogether some 150 observations of the transit of Venus were made from twenty-five countries under the aegis of the Royal Society and Nevil Maskelyne, the Astronomer Royal of those days.

Last week's observations, in which two of the telescopes were identical to those used by Captain Cook, were in part a nostalgic exercise in memory of the days when Greenwich ruled the heavens—the Greenwich meridian passes by a few yards away—and in part, no doubt, a cunning allusion to the present scandalous discrepancy between the radar and astronomical estimates of the Earth–Sun distance that Captain Cook was sent south to measure once and for all. But whether in nostalgia or satire, the clouds above Greenwich finally thinned to reveal a watery Sun, glinting down on the Dolland and Sheepshank telescopes, the Altazimuth dome, the orreries, astrolabes and armillary spheres of Greenwich. The observers hastened to their tripods, lead weights were re-hung, clockwork drives creaked into action and the image of the Sun's disk came into focus in a dozen eyepieces. There was Mercury, making a fine transit right across the middle of the disk, threading its way through a veritable rash of what the observers said were sunspots but which may have had something to do with the specks of dust on the antique object glasses.

ENGINEERING

Shaping up Ship Research

The establishment of a unified Shipping Research and Development Institute and a separate industrial training board for shipping are among the chief recommendations of a committee investigating the shipping industry in a report published last week (*Report of the Committee of Inquiry into Shipping*; HMSO, £3). The committee, set up under Lord Rochdale in 1967 by the President of the Board of Trade, emphasizes the shortage of good management in the middle echelons of the industry and comes out firmly against substantial state involvement in the industry. It rejects the need for any increase in government expenditure on research and development in shipping.

Although the report dwells for the most part on trade and manpower questions, the committee has clearly found cause to study in some depth the innovative side of the industry. For one thing, research on shipping and relevant aspects of engineering is unnaturally fragmented, the committee points out. There is no shortage of money for R and D projects—government expenditure compares favourably with that in other countries—but there is a lack of coordination within the industry, because companies are too preoccupied with safeguarding narrow commercial interests.

The establishment of a central R and D institute is the remedy proposed by the committee. The institute would embrace the Ship Division of the National Physical Laboratory, the British Shipping Research Association and the Shipowners' Refrigerated Cargo Research Association, with anyone else free to join should they wish. But despite the committee's designs it seems unlikely that the limited research of direct relevance to shipping being undertaken at places like the National Engineering Laboratory, East Kilbride, and the Hydraulics Research Station at Wallingford, will be transferred from the present sites.

The British Ship Research Association, founded in 1944 as a central research organization for the shipbuilding, marine engine and ship repairing industries, has, with government encouragement, extended its interests to include limited research on behalf of the shipowners. Of the £1·15 million research budget for 1968/9, the government contributed 34 per cent, the shipowners 7 per cent, and most of the remainder was provided by the shipbuilders. Only about a third of the £700,000 annual income of the NPL Ship Division comes from bodies other than the government. Apart from testing marine craft and ship propulsion devices the Ship Division also undertakes more fundamental research into ship hydrodynamics and propulsion and has recently increased its involvement in basic ship design.

The pattern of university research into shipping reflects academic demarcations between disciplines. Thus a project of computer aided ship design is being undertaken at Imperial College, London, under a contract from the Ministry of Technology. Naval architecture, however, derives its research funds from a range of different bodies, including the Science Research Council, the BSRA and the Institution of Naval Architects. The committee notes the little work being done at universities on engineering, equipment or systems specifically for ships, and chides the Chamber of Shipping for its lack of a university research programme. The shipowners together claim to spend about £3·6 million a year on R and D, but much of this could also be labelled production work. Only 4·7 per cent of this figure goes on automation and instrumentation and 3·5 per cent on hydrodynamics and basic ship design.

SCIENCE POLICY

Science No Longer an Opiate

In the rush to exploit the results of research and development for economic ends, social implications have often been overlooked and future developments in science are now greatly feared by an increasingly more vocal public. These fears have found expression in the growing hysteria about pollution, in demands for reductions in government support of scientific activities and even, in the United States, in a trend towards a general anti-intellectualism. This gloomy picture was painted last week by Dr Alexander King, Director-General for Scientific Affairs of the Organization for Economic Cooperation and Development, at a lecture given to the Science of Science Foundation. The remedy, he said, lies in a different approach towards the planning of science and technology. If the anti-scientific lobby should succeed in curbing the activities of science and technology, society would lose one of its most powerful weapons for solving social problems. But fundamental changes must be made in present thinking on science policy.

For one thing there must be a change of emphasis from an economic incentive for technological development to one in which the research effort becomes an instrument for improving the quality of life. This requires a high degree of cooperation between different disciplines and a herculean effort from the social sciences in particular, Dr King said. The first step, however, is to get away from the idea that economic growth is an end in itself, and to replace the index of gross national product with a measure which would include qualitative elements and data derived from social indicators. Such a shift in priorities would channel more attention to social problems as well as encouraging a clearer formulation of national goals.

Although the formulation of national goals is beyond the scope of science policy as such, Dr King suggested that the scientific approach has much to contribute through a dialogue which relates needs to new possibilities. In the lack of clearly defined national goals, science cannot redeploy its resources to meet the needs of society. But to do so requires a collective statesmanship on the part of scientists whereas too often scientists operate as separate and unnecessarily competitive pressure groups which may serve the interests of particular disciplines but are ineffectual if research is to contribute anything towards changing national objectives.

There is room for international cooperation, Dr King suggested, in assessing the social and cultural as well as the economic consequences of alternative technological developments. For example, a world wide early warning system on the environmental and social threats posed by new technological projects could help to avoid many of the disasters of present planning procedures which, Dr King suggested, "often serves merely to make situations which are inherently bad, more effectively bad".

It is in governmental and political structures, however, that many of the more fundamental changes are needed. The vertical organization that is found in most countries is unresponsive to the complex problems that confront science policy, and Dr King suggested that an integrated approach is difficult to achieve when a large number of government agencies are responsible each for a particular fraction of a major problem area. Establishing still more coordinating committees will not help. "Strategies concerning science, technology, economic and social policy must be considered in their interactions," he said, and this "would seem to require a senior minister, placed close to the prime minister but without departmental portfolio, to develop integrative and longer term policies."

MARINE EXPLORATION

Diving Saucers

SEA trials of the latest French deep diving vessel began last week off Marseilles. Built and equipped at a cost of some £375,000 to the Centre National d'Exploitation des Océans, and capable of descending to 3,000 m below the surface, the *soucoupe plongeante* (SP 3000) is one of a series of submersibles that stem from designs by Commander J.-Y. Cousteau's research group in the late 1950s. The French Navy is already interested in borrowing the vessel to photograph the remains of the submarine Eurydice which was wrecked a few months ago, and other possible uses include direct exploration of such places as the mid-Atlantic ridge as well as allowing marine biologists to plumb new depths.

Despite its name, the SP 3000 has the shape of a rugby ball. A tough fibreglass shell encloses the engine, the steering equipment and the central cabin, which is a steel sphere with room for three people; three batteries provide the power and the vessel can travel at up to 7·5 km/h, although, unlike the earlier SP 350 which propelled itself by ejecting jets of water under pressure, the SP 3000 is driven by twin screws. During its trials it is based on the oceanographic ship Jean Charcot, which seems at present to be the only ship with the facilities for launching it at sea. Plans for expanding the fleet of the Centre National d'Exploitation des Océans provide for building several smaller ships that will be able to use the SP 3000.

TECHNICAL COLLEGES

Stretching the Year

by our Education Correspondent

TECHNICAL colleges in Britain have been categorically included in the effort to improve cost effectiveness in educational establishments. In a closely argued report, the Committee on the More Effective Use of Technical College Resources has urged local education authorities to take a hard look at the colleges under their control to see what profit there may be in increasing the length of the college year. (*A Report of an Inquiry into the Pattern and Organization of the College Year*, HMSO, 3·5*s*.) The committee, which advises the Secretary of State for Education and Science, is careful to say that its recommendations must be interpreted according to the needs of each individual institution, but it comes down firmly in favour of lengthening the college year on the grounds of more efficient use of resources. This should allow new types of course to be taught, and will fit in better with the requirements of industry.

Although there has been a trend in recent years towards keeping colleges open for 48 weeks a year instead of the traditional 36 or 37, the committee points out that only a few students are taught during the extra weeks, and that very few colleges have rearranged their courses to gain the maximum benefit from the extra opening hours. For example, there is still considerable scope for running the same block release course several times a year so that it can be taken at the appropriate point in a student's industrial training, and there is no reason why the length of the year for part time students should be the same as that for full time students. A lengthening of the year for part time students, the committee suggests, could be used to broaden the content of courses, and it will also even out the load on a firm's training facilities.

The further education sector is, of course, more able to incorporate changes of this kind because of the diversity of courses which its establishments provide, but similar arguments are gaining some ground in the higher education sector as well. The government has already canvassed the possibility of introducing four term years in the universities, and it is still casting around for ways to meet the demand for places in higher education as cheaply as possible. But—

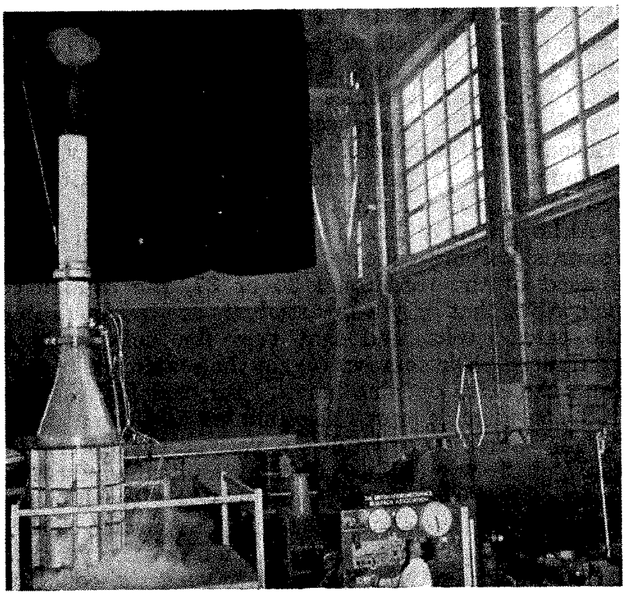
elections permitting—the education world should know in the next three or four weeks what is in store for it when the government publishes its long awaited green paper on education.

POLLUTION

Vortical Chimneys

Chimneys that blow smoke rings are not just a Dadaist's dream. A model which does just this has been built by a team under Dr P. J. Baker in the laboratories of the British Hydromechanics Research Association at Cranfield, near Bedford. Cheaper in principle than the tall chimneys that are at present used to discharge industrial smoke, the design uses a fluidic switching system to punch out smoke in discrete vortices.

The advantage of a vortex is that it can roll upwards instead of sliding through the air as a plume of smoke does and, because the friction is reduced, the smoke can ascend perhaps four times as high. Although the idea is not new it has been hard to devise machinery for generating the vortices that does not require heavy maintenance. The fluidic valve developed by Dr Baker's team diverts the smoke into a ring-shaped reservoir between puffs. A blast of high pressure air, introduced by a vertical pipe that rises through the centre of the chimney, draws the smoke out of the reservoir and with the help of the internal geometry of the chimney imparts to it a rolling motion. After the smoke ring has been expelled, a mechanical valve at the top of the chimney cuts off the smoke that remains inside and sends it back to the reservoir.



General view of model emitting smoke ring.

In the laboratory the rings last for some while, but it is not clear how they would fare in a stiff breeze. In theory their stability should depend on the ratio of the ejection speed to the wind speed, and a shield at the top of the chimney might allow rings to form fully before they are released. One teething trouble is that each puff produced by the model sounds like a blow from a steam-hammer but the use of absorbent materials should solve the problem.

The next step is to construct a larger model for testing in the open air, after which an industrialist sponsor will be needed to support a full scale trial (the National Research Development Corporation is backing the present work). The most useful habitat for the fluidic chimney is expected to be in valleys and densely populated areas where smoke near the ground is a particular nuisance.

HYDROGRAPHY

Floating Superproblems

The new breed of supertankers of over 200,000 tons, and the prospect of even larger ships up to 500,000 tons in the future, is creating a major problem for hydrographers, according to the annual report by the Hydrographer of the Navy, Rear Admiral G. S. Ritchie. Many of these vessels have draughts of more than 80 feet and there is an urgent need for detailed surveys of routes that are suitable for them. In particular, the report expresses anxiety about the minimum clearances which are accepted by the tanker companies.

The problem is that the sort of surveys which are required to make the operation of these vessels safer take time to complete and are expensive. "In the meantime," the reports says, "the construction of these monsters continues." In particular Rear Admiral Ritchie is concerned in his report about the risk to deep-draught vessels around north-west Europe. The seas in this crowded area are shallow and changeable, and cluttered with the wrecks of two world wars. To make matters worse, there is apparently a tendency to allow supertankers to be used in critical navigational conditions so as to get the most use out of expensive capital assets.

This is why Rear Admiral Ritchie says it is important to chart the deep water approaches to the British Isles, in particular the southern North Sea westwards of 3° E, which delineates the approaches to the Thames Estuary and the English Channel. The same applies to the Persian Gulf and the Malacca Strait between the Malayan Peninsula and Sumatra, all on important tanker routes. An additional hazard which crops up in the North Sea and the Malacca Strait is the presence of what are called sand waves which often shift their positions and can be up to 30 feet in amplitude. This is a problem which the Natural Environment Research Council is investigating.

Rear Admiral Ritchie points out that the charting of the Persian Gulf and of the North Sea is a British responsibility. An international effort involving Britain, Japan, Indonesia, Malaysia and Singapore is dealing with the Malacca Strait.

EXHIBITIONS

Peering into the Micro Era

The international Instruments, Electronics and Automation Exhibition that opened at Olympia last week was billed as the largest exhibition of its kind ever to be held in Britain. Certainly a varied collection of genuine innovations was on display, together with instruments the style of which was not always perceptibly different from those of earlier years.

Automatic Systems Laboratories displayed a high precision thermometer capable of measuring to better than one millionth of a degree at 1 K, a device developed in conjunction with the National Research Development Corporation. EMI took the occasion to promote its impressive array of electron microscopes and microanalysers, and Thorn Colour Tubes Ltd set the seal on the colour television era by advertising its intention of manufacturing more than 300,000 colour television sets a year from 1971 at its new factory in Lancashire.

An interesting exhibit in nuclear engineering was an automatic beta gamma spectrometer, made by Nuclear Enterprises Ltd, with facilities for liquid scintillation and gamma counting and an automatic sample changer. Another attraction was the Computer Technology's "fun palace", in which visitors were invited to pitch their gaming skill at one of Computer Technology's new modular computers.

One exhibit with special appeal was the S-band power amplifier which was used to boost the television, voice and telemetry signals from the Moon. An important feature is the redundancy built into the equipment to guard against the failure of any component.

PESTICIDES

DDT Safe but Resistible

In the last few months resistance to insecticides has been growing almost faster among people than insects and the usefulness of the chemicals is becoming seriously eroded. As far as biological resistance is concerned, a hundred species of insect have become immune to DDT between 1962 and 1968, compared with the 81 species that developed resistance in the previous six-year period, according to a report compiled by the World Health Organization's Expert Committee on Insecticides (*Insecticide Resistance and Vector Control*, WHO, Geneva 1970. £1·25; $4).

According to a questionnaire sent to public health authorities throughout the world, the insecticides that are chiefly used in the control of disease carriers are the organochlorines DDT, HCH and dieldrin. But a growing number of insect species have developed resistance to one or more of these chemicals. In many places resistance has been acquired by the principal malaria vectors, and in West Africa DDT and dieldrin are essentially useless.

On the effect of DDT in the environment the WHO committee seems to have shifted its ground a little from its statement in 1967 that "the safety record of DDT remains outstanding . . .; the concern . . . about contamination of the environment by this very stable and persistent insecticide should not . . . be considered sufficient reason for substituting other insecticides". Rather more concern is shown now, but although the committee recognizes the desirability of using less persistent compounds it still feels that DDT must continue for some time yet to be a major insecticide.

The present concern about environmental contamination has led to restrictions on their use and to support for more biologically orientated research. Genetic control has received much of this attention and encouraging results have been achieved with sterile male techniques including those based on naturally occurring sterility mechanisms such as cytoplasmic incompatibility and hybrid sterility. The latter uses hybrid crosses which produce males which are sterile but show increased competitive mating ability because of hybrid vigour.

But although the use of chemical insecticides on an empirical basis is beginning to give way to biological techniques based on a more fundamental understanding of insect biology, the committee emphasizes that control must continue to rely on chemical pesticides for the control of insect and rodent vectors of disease; its opinion is that it is not realistic to expect that alternative methods will become available in the near future.

CHEMISTRY

Forced Changes

An investigation into the way that chemistry is taught in British universities is to be conducted at the University of Liverpool. The university has been given a grant of £7,000 for the study by the Leverhulme Foundation, and it is expected to take about three years to complete. The main incentive for the investigation, it seems, is that universities are finding that they have to adapt more and more to changing patterns of teaching in the sixth forms, and the pressure for change at university level will become even more acute if the proposals for "Q" and "F" level examinations are accepted in July by the Schools Council and the Standing Committee on University Entrance.

Professor A. K. Holliday of the Department of Physical Chemistry at Liverpool and Dr W. J. Hughes of the School of Education, who will jointly supervise the investigation, believe that by 1972 most students entering university to read chemistry will have a very different educational background from present entrants. Acceptance of the Nuffield sixth form science courses and the more liberal A level syllabuses being introduced by some examining boards—especially London and the Northern Universities Joint Matriculation Board—means that many will not have read chemistry as a separate subject and their knowledge of the basic facts will be less firmly grounded although they should be better acquainted with the important principles. Moreover, students who have taken the carefully planned new school curricula will expect their university courses to be equally well thought out.

The investigation will be concerned in the long term with the effectiveness of the whole teaching system, including lectures, tutorials, laboratory work and the assessment system. In the short term, it will look at changes in laboratory courses. The study is claimed to be the first of its type, which is surprising because it deals with the kind of problems facing every chemistry department. But fortunately one of the conditions imposed by the Leverhulme Trust is that the results of the investigation must be made freely available.

The proposals for Q and F level examinations and the accompanying changes in sixth form curriculum which would call for even greater changes in the universities now seem to be running into mounting opposition. A group of Leicestershire teachers have started a movement aimed at persuading the Schools Council and the standing committee on university entrance to reject the proposals when they come up for acceptance in July. The main complaint is that the proposals could lead to three examinations in three years, and that they will not necessarily broaden sixth form curriculum.

Parliament in Britain

Defence Research Contracts

THE Ministry of Technology expects to spend about £1 million during 1970–71 on university agreements sponsored by its research and development establishments. About £250,000 of this will be spent on wholly civil projects for the industrial research establishments, and the rest, which relates to agreements sponsored by the aerospace establishments, consists chiefly of research which has both civil and defence applications. Dr Ernest Davies, Joint Parliamentary Secretary, Ministry of Technology, who gave this information in reply to a question from Mr James Dickens, said that it is not yet possible to say how the total amount will be apportioned between the various universities.

Pollution of Scottish Waters

ABOUT half of Scotland's effluents are discharged into the Firth of Clyde, and about a quarter into the Firth of Forth. There is pollution on the West Coast from iron and steel manufacture, from coal, sewage sludge, paper pulp, sugar refining, power station wastes and from the dumping of explosives and sometimes of explosive wastes. This dismal picture was painted by Dr Dickson Mabson, Minister of State, Scottish Office, in reply to a debate initiated by Mr John Rankin, but Dr Mabson could give no assurances that fresh legislation would be brought in to deal with the situation. The Clyde River Board, he said, spends about £150,000 a year dealing with pollution of that river, and the white paper which was promised when the Royal Commission on Pollution was set up may lead to controls in areas where there is an obvious problem. (Debate, May 6.)

CS Gas

THE government has no intention of reconsidering its policy towards CS gas in the light of evidence from tests carried out by the United States Army. Mr Stewart said that he has examined the evidence carefully and that it contains nothing to upset the government's view that CS cannot be regarded as significantly harmful. The Foreign Secretary did not agree with Mr Frank Allaun that if Britain excludes CS from the Geneva Protocol, that may encourage other nations to remove their pet weapons from the ban. (Oral answers, May 4.)

University Salaries

THE government has given its approval to new machinery for negotiating the salaries of university teachers. The first stage of the negotiations is for the Association of University Teachers and the university authorities to agree on a claim, which would then be put to the government as the paymaster. There is also provision for arbitration if final agreement cannot be reached. Announcing the government's agreement to these proposals, which had been put forward by the Association of University Teachers, the University Grants Committee and the Committee of Vice Chancellors and Principals, Mr Gerald Fowler, Minister of State in the Department of Education and Science, said that the salaries of clinical staff will continue to be related to those of hospital doctors and dentists, and they will be determined by the government in the light of recommendations put forward by the Review Body on Doctors' and Dentists' Remuneration. The setting up of this machinery means that the university salaries will not come under the control of the Commission for Industry and Manpower. (Oral answers, May 7.)

Miscellaneous Intelligence

AN article in a recent issue of *Physical Review Letters* is entitled "High-momentum-transfer neutron-liquid-helium scattering Bose condensations". This equally-straining-to-mind-and-eye nearer-to-Teutonic-syntax-than-English demeaningly-tortuous-way-to-commune-with-even-one's-professional-colleagues stylistic solecism is one that can be taken too far.

THE Hydrographic Department of the Ministry of Defence has raised from 1*s* to £1 its minimum charge for answering public enquiries about wrecks, a 20-fold increase on which even treasure hunters may founder and the Prices and Incomes Board sink without trace.

DID any Romeo ever sigh beneath his Juliet's balcony and proclaim his "affiliative need, mutual absorption and desire to promote another's (*scilicet* Juliet's) welfare"? Even if not, that is what he meant to say, if the verdict of the American Association for the Advancement of Science is anything to go by. The AAAS has awarded its annual Socio-Psychological Prize to a sociological thesis on romantic love, love being defined in such terms for the purposes of the study. The purpose of the prize is "to encourage in social science research the development and application of dependable methodology on the lines of the natural sciences".

MANUFACTURERS of cyclamates in Britain and the United States may not have liked the ban on the artificial sweeteners imposed last year, but at least they lay down under it. Not so in Japan where the Kanto Chemical Sweetenings Industry Association and the East Japan Association of Producers of Artificial Sweetenings have sued the government for $500,000 to cover the losses incurred from the ban. The associations claim that the government studied only the research carried out in the United States and failed to collect data in Japan, whereas it is required by the food sanitation law to use the findings of research conducted at more than two places.

MRS CHERUBINA ALLIEGRO of Naples has attained the 108th year of her age, which she attributes to never having taken any medicine.

THOSE who complain that scientists, even physicists, write with too little confidence will be cast down by the following passage:

"We choose not to review each of the developments in detail here, but wish only to state that in our opinion no one of these authors has derived the most general and complete set of results that is achievable, nor has used as simple and self-contained a formalism as possible.

"In this paper we attempt to collect the results of the previous authors on the self-consistent phonon theory; to correct some minor errors; to extend the results to more general situations in several directions; to correlate these results by deriving them all from a single formalism; and to select a formalism for their statement which we feel is clearer and more compact than others, while no less powerful. The functional variation technique which we employ, while not entirely novel, at least has not been used previously in the study of lattice dynamics for which we feel it is ideally suited."

No prizes will be awarded for identifying the source.

NEW WORLD

Another Echo of Received Doctrine

THE most recent public complaint about the condition of basic science in the United States has popped up within the White House itself in the form of a report of a Task Force on Science Policy under Dr Ruben F. Mettler, president of TRW Inc. The committee was set up last October with a request from the Administration for guidance "for 1970 and beyond". In practice, although the task force has added its voice to those who plead for a stronger role for the National Science Foundation in the support of basic science, and although it takes up the long forgotten theme of the need that the Federal Government should acknowledge the support of graduate education to be a federal responsibility, its report will not help to distinguish between the more radical proposals for change put forward by other bodies.

The task force has tried to spell out the reasons why science and technology are important. It argues that vigorous programmes in science and technology are necessary in fields as different as national defence, arms control and the improvement of personal health. It shakes its head over "the rapid rise of attitudes disdainful of science and technology" and says that the solution is better public understanding of both the limitations and the promise of science and technology. The task force also considers that "leadership and excellence in science" should be, for the United States, an objective in its own right. There follows from this, the argument goes, a need to balance regional and national interests within the United States and also a need that scientific techniques should be used to predict the evolution of social problems and their solution. Above all, science and technology are essential for economic growth and the task force seeks to make this point by quoting Mr Kosygin's view, in March 1966, that the outcome of the competition between "the two world systems" would be determined by scientific development. What the task force asks is that the President should affirm in public that the development of science and "continuing leadership" in science are important national roles.

The task force argues that several things can be done to make better use of science and technology and to make these uses seem more relevant. This part of the report will no doubt bring comfort to the National Science Foundation, also now beating this drum (see *Nature*, **226**, 495; 1970). One field in which better use might be made of existing skills is in the development of health services—"we have failed to devote sufficient resources to defining the ways in which existing technology can improve health care and at the same time help control its cost". But the task force also asks that the social and behavioural sciences should be given more support and more scope for dealing with practical problems and suggests that there should be programmes of development towards "a system of social indicators", a national data system for use in the social and behavioural sciences, graduate schools of applied behavioural science and more money for research in these areas, long term and applied. The report suggests that the National Science Foundation and the Office of Science and Technology should between them take the initiative. It also pats on the back the several groups, from the Daddario committee and the National Academy of Sciences to the National Academy of Engineering, who have recently given the name "technology assessment" to the process of trying objectively to decide whether particular pieces of technology have a valuable role to play in the solution of social problems.

What this boils down to is the recommendation that departments of the Federal Government of any size should be asked to develop, and then to maintain, ten or fifteen year projections of their long term plans in such detail that the consequences can be anticipated by planners and scientists alike. As the task force sees it, the Office of Science and Technology should play the central role in coordinating these plans and trying to identify the points at which scientific and technical people could help to ameliorate the consequences of change.

The essence of the task force report lies in its recommendations on the machinery for supporting basic and applied research with federal money. The committee says that current problems have emerged only because expenditure has stagnated or even declined. Indeed, in the past four years, when the level of government support has remained for practical purposes constant, the committee estimates that inflation has decreased the real resources available by a quarter—a calculation which takes no account of the increasing cost of scientific research. But, the task force says, the universities are still turning out young people previously encouraged to work in science and who now find it hard to obtain jobs "consistent with their interests", while the mission agencies, under pressure from their own budget, have abandoned research in several areas of science—the Office of Naval Research has pulled out of nuclear physics, the Advanced Research Projects Agency has retrenched in material sciences, and the National Institutes of Health are no longer supporting chemistry and plant science on the old scale, so that the balance of American science has been distorted. The task force complains that the National Science Foundation is not in a position to take over projects abandoned by the mission agencies, that "mature and productive investigators in all disciplines" are finding it increasingly difficult to carry on their work and that universities are embarrassed because they cannot meet obligations sensibly assumed in times when money was easier to find.

What is to be done ? What the task force suggests is that "the time has come for more effective commitment to long range research" and that the National Science

Foundation should become the chief government agency for this work, with the National Institutes of Health following close behind. The task force says that the cost of supporting basic research will continue to increase with the national economy, and suggests that the budgets of the National Science Foundation and the National Institutes of Health should be tied to the Gross National Product. The argument is that increasing prosperity will bring proportionately increasing demands for improvement in urban planning, health care, education and the like.

Graduate education plays a central part. The task force says that there have been so many studies of this issue that the Federal Government should by now know enough to take effective action, and it commends the spirit of the first report of the National Science Board, which, at the beginning of 1969, asked for a federal commitment to graduate education and for a new pattern for distributing funds to universities based on a variety of novel types of grants to university departments as well as traditional grants for research projects. Lending further support to the wishes of organizations such as the Daddario committee and the National Science Board, the task force also asks for fuller use of federal laboratories and indeed goes as far as to say that the Federal Government should discourage "completely self-contained capabilities within mission oriented research institutions".

But how should these questions be coordinated? The task force asks for a more explicit concern for the comparatively small proportion of the federal budget for research and development which finds its way into basic research—a mere nine per cent of the $17,000 million a year at present being spent. The difficulty under the present arrangements, according to the committee, is that for lack of coordination within the Federal Government, decisions between different fields of science are being made without a balanced judgment of all the pros and cons. The task force asks that the Federal Council for Science and Technology should take responsibility for this kind of judgment, chiefly because this is the only instrument of the Federal Government likely to persuade the agencies to do what is expected of them.

In practical terms, the task force would like to see the budget of the National Science Foundation increased as rapidly as possible to 0·1 per cent of the Gross National Product. At this level, the National Science Foundation would be responsible for roughly one third of all basic and academic research supported by the Federal Government, but it goes on to say that several years may be needed to reach such a condition. The committee's report also wishes to see the National Science Board's report on graduate education turned into law not later than a year from now, a survey of the federal laboratories to see what better use might be made of them, and the confirmation of the Office of Science and Technology as the chief instrument for negotiating priorities in basic research.

The committee echoes several recent pronouncements on science policy by asking that there should be more research within the military programme "even at the expense of current military hardware procurement" if that should be necessary, more encouragement for "new science based foreign policy initiatives and opportunities for international cooperation" and a continuing interest in the development of science policy as such.

TASK FORCE REPORT

No Immediate Prospect

The reaction of the Administration to the publication of the Task Force Report seems to hover between pleasure and embarrassment. There is, of course, no time for the report to have an influence on the budget process for the coming year, now well under way. The most likely development is that what the report has to say will in due course influence the budget proposals of the National Science Board (on behalf of the National Science Foundation) due to emerge in February next year.

One possibility is that the National Science Foundation may then be seen to be asking that more of its funds should be distributed according to the pattern originally suggested at the beginning of 1969, although there is every likelihood that the National Science Board itself is by no means clear in its own mind about the desirability of this new pattern, which would spend a greater proportion of the money needed for basic research in the form of grants to departments and students not linked with specific research proposals. With Dr Philip Handler, the present chairman of the board, likely to leave later in the summer, it is on the cards that the board will want to think again about its commitment to this proposal.

Whether the National Science Board will be encouraged by the Administration to a more ambitious view of its responsibilities is also problematical—certainly there is no immediate sign of a public declaration by the President about the importance of science and of graduate education—this would no doubt seem a little like signing a blank cheque. The real test will be the extent to which the National Science Foundation is encouraged to bid for more money twelve months from now.

On the larger question of the form of organization needed for planning the development of science and technology, the task force has avoided reference to some of the more daring schemes that have recently been talked of—the proposal for setting up the National Institutes for Research and Advanced Studies put out by the Daddario committee some weeks ago, for example. But, on the other hand, the task force has branched out vigorously on its own with the proposal that the Federal Council for Science and Technology should be given the power to coordinate the scientific work of the mission agencies even to the extent of making sure that Federal Government laboratories are properly integrated with the rest of academic life. Few agencies will welcome this proposal, even though there is no obvious reason why the federal council should not undertake this kind of coordination without formal direction from above.

UNIVERSITY OF WISCONSIN

Looking for a President

from a Special Correspondent

THE latest to join the long list of institutions seeking new presidents is the University of Wisconsin, a huge land-grant multi-versity with sixteen campuses and a liberal tradition centred in an essentially rural state. Only a few hours after he had returned to Madison from Washington, where he and seven other "élite university" presidents tried to persuade President Nixon to cool the Administration's attitude towards students, Fred Harvey Harrington surprised nearly everybody by announcing his resignation.

There was a bit of irony in Harrington's resignation. He is a recognized authority in the field of American policy in the Far East, and has been an adviser in this area to Presidents Eisenhower, Kennedy, Johnson and Nixon. He gave up his $47,000 a year job as president to become a $35,000 a year history professor, effective from October 1, on the fourth day of rioting on the campus, touched off by the decision to expand the war into Cambodia. In recent years, Harrington had clashed often with some of the regents and with several state legislators. One state senator from a northern rural area of Wisconsin carried on a campaign against the university's president and passed out automobile bumper stickers reading: "Harrington Must Go". Generally, Harrington was popular among the students. Several regents urged that he take a tougher line on students although they were unable to define exactly what they wanted. Harrington told a news conference that his resignation had been "long-planned" and he denied that he had been dismissed. "The timing is accidental," he said.

In spite of everything, Wisconsin is viewed by many as one of the great universities. It is especially strong in the physical and the biological sciences. It has been the home of two recent Nobel laureates, Joshua Lederberg and H. Gobind Khorana. But Stanford stole Lederberg and the Massachusetts Institute of Technology lured Khorana after they received their prizes in Stockholm. Tight budgets, enforced by the legislature and the regents, have made it difficult for administrators at Wisconsin the last two years. In addition, a strict quota restriction on students from out-of-state has been adopted; there have been allegations that these quotas are aimed at prospective students from such states as New York, New Jersey and Connecticut which, through the years have been sending their "Harvard rejects" to Wisconsin. Some have even suggested that these restrictions are aimed, directly or indirectly, at sons and daughters of Jewish families because quite a number of out-of-state students at Wisconsin are Jewish.

Harrington graduated from Cornell University and took a doctorate from New York University in 1937. He came to the Middle-West and, for a number of years, was chairman of the department of history at Wisconsin before his nomination to become president. He was a leader in the formation of two middle-western consortiums for higher education—the Argonne Universities Association, which is the policy-making body of 39 institutions concerned with the long-range plans of the Argonne National Laboratory, near Chicago, and the Committee on Institutional Cooperation, concerned with inter-disciplinary studies, mainly in the sciences, linking nine public and two private universities from Ohio to Iowa.

Now that Harrington is "out", it will be especially interesting to see who will become the fifteenth president of the University of Wisconsin. The tradition has been to find, if possible, a man within the system rather than to go outside. One possibility is another historian, Irvin G. Wyllie, the scholarly chancellor of the university's new Parkside campus near Kenosha. He has been building a new campus and a new faculty and, to date, has not been scarred by the student revolt.

To California without Smoke

THE strangest by-product of the campaign against pollution, the Clean Air Car Race between Cambridge, Massachusetts, and Pasadena, California, seems destined to crowd the transcontinental roads even more tightly this year, in the week of August 24. So far, 35 entries have been registered, and it is expected that there will be further entries from at least two university teams anxious to explore the virtues of liquid hydrogen and liquid oxygen as propellants for road vehicles. So far, however, most interest centres on a mystifying entry from the University of South-eastern Massachusetts, whose entry is said to require no fuel and no supply of energy at stopover points. Speculation ranges from gyroscopically driven vehicles to wind-driven road yachts, although the fixed route prescribed for the race will presumably prevent the kind of tacking that would make arrival at the California Institute of Technology an uncertain process.

The two originating colleges, MIT and Caltech, will be responsible for testing all entries for safety before they take the road. The previous race, in 1968, involved two electrically driven vehicles racing in opposite directions across the United States. This year, both MIT and Caltech (which won last time) will enter two comparatively clean vehicles. The MIT entries include a car with batteries which are charged intermittently by means of a petrol driven engine—the device is expected to be less polluting than a simple internal combustion engine—and a turbine driven electrical generator driving an electric motor which will weigh 225 pounds and generate 45 kW amperes. The second of these two cars breaks new ground by using an electric drive motor capable of delivering up to 600 horsepower and weighing close on half a ton. Although the turbine should produce much less pollution in its exhaust than a conventional engine, there will still be some nitrous oxide.

The occasion of the race has provided General Motors, recently much maligned by student groups, with an opportunity to contribute twenty new cars and $40,000 to the committee responsible for the race. The largest entry is expected from the Worcester Polytechnical Institute, which is expected to enter six cars, some driven by steam and some by electricity.

SCIENCE BUDGET

Unaccustomed Gentleness

THE Appropriations Committee of the House of Representatives has this year dealt more leniently with the National Science Foundation than its practice in the past few years had led many people to expect. The National Science Foundation budget for the year beginning in July, if the Appropriations Committee has its way, will be $497 million, $16 million less than the sum of $530 million asked for in the Administration's budget. These sums of money include both the allocations for the Sea-Grant colleges, intended to stimulate research in oceanography, and the comparatively small sum spent by the National Science Foundation outside the United States. In its recommendation to the House of Representatives, the Appropriations Committee says that it approves of the National Science Foundation's growing interest in problems of a practical character—the environment for one. It does, however, also specify that the level of expenditure on summer institutes should be maintained at its present level—equivalent to $23·3 million—and not cut in half as the Administration has suggested. This means that the National Science Foundation will have less leeway in its planning than the comparatively small overall reduction would suggest, but there has nevertheless been a good deal of relief, both at the National Science Foundation and the Office of Science and Technology, that there will be an increase of something exceeding $57 million in what the National Science Foundation has to spend next year.

By all accounts, the Appropriations Committee considers that an increase which amounts to more than 12 per cent from one year to another must surely be sufficient. In practice, the budget will eventually be determined by whatever compromise is reached between the Appropriations Committees of the Senate and the House of Representatives, while the amount of money which the foundation has to spend will be determined by how the Administration chooses to live within the limits set by Congress. There is, however, a good chance that the Senate will take a more generous view than the House of Representatives has done, so that even though the budget may not be as great as the optimists expected, it may eventually turn out to be a little better than at present.

HELIUM

Intimations of Mortality

ONE of the unsung casualties of the impending restriction of federal money is the Helium Research Center at Amarillo, Texas, a laboratory of 65 souls set up in 1960 as part of the Federal Government's Helium Conservation Program. According to an official of the programme, which has only recently been set up independently of the Bureau of Mines (on April 1), it will be possible to employ 14 of the people displaced in other parts of the conservation programme, especially on the analytical side. The laboratory as such will, however, shut down at the end of the financial year on June 30, chiefly because of the vigorous steps being taken by the helium programme to economize on its budget, which at present amounts to $50 million a year, and partly because of the view, disputed by some of those at the laboratory itself, that the laboratory's work is only indirectly relevant to that of the helium programme itself. In fact, the centre has been concerned with the thermodynamic properties of helium and mixtures of helium and nitrogen—matters of great importance in determining how helium bought under the conservation programme should be paid for, the development of analytical techniques and the preparation of exceedingly pure quantities of the gas for physical work. By all accounts, the decision to close the laboratory is extremely sudden—indeed, the federal budget for 1971 includes $1·3 million for the laboratory, evidently now to be saved.

The closing of the laboratory will draw attention yet to the continued difficulty of operating the Helium Conservation Program, said to have been under review for the past year. At the helium office earlier this week, an official said that attempts were being made to save money not merely by closing down the Helium Research Center, but by economizing on headquarters staff and by consolidating the cryogenic operations involved in the production of helium at the Exell plant in Texas. What has forced the programme into difficulties is the failure of the demand for helium to grow as quickly as had been expected some years ago, when prices were fixed for the purchase and stockpiling of helium extracted from the natural gas of Texas, a unique resource in which helium may constitute between 0·4 and 2 per cent of total volume. In the present decline of the market, it has been possible for private operators to undercut the government's selling price of $35 per thousand cubic feet by up to a third, in the process winning a dominating place in the private helium market.

The principle on which the Helium Conservation Program operates is that the government buys at $12 per thousand cubic feet and sells at $35 per thousand cubic feet, stockpiling the gas in underground reservoirs and planning to recover the cost by eventually selling off the helium to what is expected to be a growing market. The original calculation, in 1960, was that the programme would liquidate itself in 35 years. In practice, demand for the gas has not increased as quickly as expected, while the government's share of the market has been restricted by the activity of the private operators to the public domain. In ten years, the Federal Government has spent close on $300 million on buying and storing helium. Over the years, the cost of this investment has increased with the cost of money, which is another reason why the programme should now be regarded quizzically. It is, however, hard to see how the Federal Government can wash its hands entirely of helium conservation—it is reckoned that something like 60 per cent of all the helium emerging from the ground in Texas finds its way into the hands of the conservation programme or of the private operators. From the National Academy of Sciences to the Office of Science and Technology, with the US Navy included, the government would be hard put to remain in the helium business. Its most likely response, in the present financial climate, would be to find some way of driving up the selling price for helium, either by outbidding the private companies for supplies or by the old fashioned but direct approach of creating a monopoly.

UNIVERSITIES

Moderation at Boulder

from a Special Correspondent

Boulder, Colorado

A NEW moderate trend is discernible among protesting students. This has been particularly well exemplified on Colorado campuses in the past week. During an "official" two day strike at the 20,000 strong university of Colorado at Boulder, the university's president, Dr Frederick Chieme, was given an ovation when he addressed a rally packed by half the university population (10,000). The students were calling for a complete shutdown of courses for the rest of the term. The object apparently is to conduct house to house teach-ins of the uncommitted "civilian" community. The president proclaimed that he was "bitterly opposed" to the war in Vietnam and for this reason was against closing the university "so that we can make our voices heard".

The moderation of the manifestations, despite the undoubted strength of student feeling there can, in part be attributed to the wise handling of the crisis. The university has a famous sociology department, and the departmental head, Professor Howard Higman, is chairman of the staff executive committee and vice-chairman of the university's governing body. An analysis of options open to the university government showed seven possibilities, ranging from severe penalties for students missing class to total capitulation—that is, shutdown of the campus.

The committee has set its face against bringing in the police, much less the National Guard, under almost any circumstances. After lengthy debate, the council voted 33 to 5 for "business as usual", with a lenient line on grading those who do not attend classes. This week will show what the student response is. Some face loss of degrees if they are not graded or examined this term, which ends anyway in three weeks' time. At other nearby institutions, moderate students have been physically interposing themselves between the authorities and extreme groups bent on violence. A line of passive defenders of their own people has in some cases been enough to turn back student wreckers.

Midweek, there was a much smaller turn out of striking students in Denver itself. A demonstration only a few hundred strong halfmasted the stars and stripes on Capitol Hill and tucked a red flag under the arm of the Union soldier on the Civil War memorial in front of the Capitol building. There was almost a holiday mood about the affair. There was also an air of family picnic prevailing at the start of the massive and highly organized Saturday rally on Capitol Hill. A march of up to eight or nine thousand students passed off without incident, largely due to excellent marshalling by student organizers themselves. They meticulously observed the police regulations for the march that demonstrators should keep the sidewalks clear. Legal marshals from Denver Law School mingled with the marchers and on Capitol Hill (where the crowd reached 12,000) to maintain discipline and to advise. A briefing prepared by the law school marshals and circulated in advance clearly set out to the demonstrators their rights and obligations and what to do if "busted". "If tear gas is used—don't rub your eyes," it concluded. A contingent of 200 medical students, both girls and boys, provided volunteer Red Cross units equipped with walkie talkies and placed at strategic points as much to set an example and head off trouble as to mop up casualties afterwards. There were none. The speakers' microphone at the Capitol was briefly taken over to preach revolution but was soon returned to the hands of the organizers. The police actually supervised the lowering of the national flag as the acting governor (Hogan) had given permission for this. Four objectors were put aside. The only arrest occurred after the scheduled meeting was over when extremists picketed the police station and refused to move from a busy street crossing where they stayed on and staged a sitdown in the road.

CANADIAN ASTRONOMY

Boost for New Institute

THE new Interdepartmental Institute of Astronomy and Space Science at the University of British Columbia, Vancouver, has been given a good start by the National Research Council of Canada in the form of a grant of $538,600. The intention is that the institute should work closely with the Astrophysics Branch of the Radio and Electrical Engineering Division of the National Research Council. This should give the institute easy access to the two major observatories in British Columbia—the Dominion Astrophysical Observatory at Victoria, and the Dominion Radio Astrophysical Observatory at Penticton—which are managed by the Astrophysics Branch.

It seems that the Vancouver institute will specialize in radio astronomy at centimetre wavelengths and less, and in plasma physics. Radio astronomy at short wavelengths is hampered by the presence of water vapour, but there are sites in Western Canada which ought to be suitable because of their height and the coldness of the atmosphere. One of the projects is to develop a 15 foot dish aerial which has an accurate enough surface to work at wavelengths of a few millimetres. The intention here, of course, is to break into the new branch of radio astronomy which deals with the detection of molecules in interstellar clouds by the short wavelength radio waves that the molecules emit. This work will be under the direction of Dr W. L. H. Shuter, associate professor in the Department of Physics. Shorter wavelengths in the no-man's-land where radio and infrared wavelengths merge will be the responsibility of Dr M. W. Ovenden, professor of astronomy in the Department of Geophysics, and part of the effort here will be in developing more efficient detectors.

The third area which will be dealt with at the new institute, plasma physics, is to be covered by Professor R. Nodwell of the Department of Physics.

The grant comes under an NRC scheme to encourage new, and especially interdisciplinary, research centres. Spread over three years the grant will be $315,800 in 1970–71, $144,800 in 1971–72 and $78,000 in 1972–73.

NEWS AND VIEWS

Making Proteins in Higher Cells

MOLECULAR biologists who have started to elucidate the mechanism of protein synthesis in nucleated cells are in the envious or invidious position of knowing what the results of their experiments should be because they already know in detail how bacteria make proteins. The synthesis of protein in microorganisms and eukaryotes is certain to be basically similar, but there are bound to be some differences. Until recently two technical problems have bedevilled attempts to sort out the similarities and differences; the first was selecting a eukaryote system amenable to precise analysis; the second was devising experiments which take advantage of available knowledge of bacterial protein synthesis but which do not preclude the possibility of revealing the idiosyncrasies of eukaryote cells. The disarray among groups studying the initiation of protein synthesis, which has been revealed in *Nature* (**226**, 414; 1970, and this issue, pages 607 and 610), proves how real these difficulties have been.

In bacteria, initiation of protein synthesis depends on the incorporation of formyl-methionine, a methionine residue with its amino group blocked by formylation. The formyl-methionine is carried to the ribosome by a unique species of transfer RNA which can both recognize the initiator sequence, the capital letter in a messenger RNA, and allow the formylation of the methionine residue it carries. Once the protein has been made in the cell the formyl group is always removed enzymatically, and sometimes the methionine residue is cleaved as well.

But which of these steps in initiation are universal and which are peculiar to bacteria? Is a blocked amino group the key characteristic of an initiator and is methionine always the initiating amino-acid? Two weeks ago (*Nature*, **226**, 414; 1970) Liew and his colleagues reported experiments which suggest that the f2a histone proteins in rat liver are initiated with an acetyl-serine residue. They injected rats with labelled acetate and labelled serine and were able to isolate histones apparently containing acetyl-serine, which could only be at the beginning of each histone polypeptide chain. This suggests that acetyl-serine can initiate the synthesis of histones, in which case a blocked amino group would seem to be the crucial characteristic of an initiator.

In this issue of *Nature* (pages 607 and 610), however, Smith, Marcker and Brown report more clear cut experiments leading to a different conclusion. Marcker's group is exploiting an *in vitro* system in which RNA isolated from EMC virus, or synthetic messenger RNA, is faithfully translated by cellular machinery extracted from Ehrlich ascites cells (*Nature*, **225**, 184; 1970). Like the familiar bacterial cell-free systems on which it was modelled, the ascites cell-free system can be precisely characterized and many of the ambiguities inherent in all experiments involving animals or cells, including those of Liew *et al.*, can be eliminated.

Smith and Marcker (page 607) have now detected two species of methionine transfer RNA in Ehrlich ascites cells, mouse liver and yeast which seem to be precisely analogous to the two species of methionine tRNA in *E. coli*, one of which is the specific initiator tRNA. Like its counterpart in bacteria only one of the two eukaryotic methionine tRNAs incorporates methionine in the initiating position when the Ehrlich ascites cell-free system is primed with synthetic messengers of known composition. The second species of methionine tRNA in nucleated cells, again like its bacterial counterpart, incorporates methionine internally into growing polypeptide chains. Furthermore, the initiating methionine tRNA in eukaryotes, but not the species responsible for incorporation of methionine internally, can be formylated by the formylation enzymes of *E. coli*. Surprisingly, however, eukaryotic cells do not contain transformylases and the initiating methionine is not formylated. A blocked amino group cannot therefore be a prerequisite of an initiating amino-acid.

The most compelling interpretation of these findings is that methionine is the universal initiator of bacterial and eukaryotic protein synthesis, and that the only significant difference is that the methionine residue is formylated in bacteria but not eukaryotes. And because few eukaryote proteins begin with methionine and at least one EMC viral protein made *in vitro* begins with alanine, the initiating methionine residue is presumably cleaved more often from completed eukaryote proteins than from completed bacterial proteins.

There are, of course, as Smith and Marcker point out, two other interpretations of their results. The initiator methionine tRNA could be one of several tRNAs specialized in eukaryotes for initiation. Alternatively the methionine tRNA might simply act by fixing the reading frame without the methionine residue being involved in formation of the first peptide bond. In this case initiation would depend on the methionine tRNA but the polypeptide chain could begin with any amino-acid with or without a blocked amino group. Both these models, however, seem destined to be short-lived. Brown and Smith (page 610) have all but eliminated the first. They find that, at low concentrations of Mg^{2+}, of some twenty synthetic messengers tested only that with the AUG methionine codon near its beginning can be translated in the ascites cell-free system, although the cell-free system contains all species of tRNA. The Cambridge group is therefore convinced that the methionine tRNA will prove to be the unique initiator in eukaryotes.

Although these experiments show that AUG at the start of a synthetic messenger is read, with methionine incorporated as the first amino-acid of the polypeptide, it is conceivable that with natural messengers the initiating methionine tRNA merely fixes the reading frame. This seems unlikely, however. Already it is rumoured that groups in Britain and the United States are finding that haemoglobin made on reticulocyte polysomes begins with a methionine residue, which in the cells is subsequently cleaved. Furthermore, Sherman and his associates reported last year that cytochrome *c* made in certain mutant strains of yeast has a methionine in the N-terminal position, which is subsequently removed to expose the normal first amino-acid, threonine. The initiating methionine codon in eukaryotes seems, therefore, to be read as it is in bacteria.

The striking parallels between the initiation of protein synthesis in bacteria and nucleated cells apparently involve more than the use of methionine as an initiator. As Prichard and his colleagues reported last week (*Nature*, **226**, 511; 1970) there are three initiation factors in reticulocytes, similar to the three initiation factors in bacteria, which are required for initiation of synthesis of haemoglobin. Although the precise details of how each of the three mammalian factors functions have yet to be elucidated nobody will be surprised if they prove to play identical parts to the f1, f2 and f3 factors in *E. coli*.

LYMPHOCYTES

Cooperating Populations

from a Correspondent

THE interesting phenomenon of cooperation between two populations of lymphocytes in the immune response has been the topic of two recent meetings. A British Society for Immunology colloquium on "thymus-derived cells" was held at the National Institute of Medical Research, London (NIMR), on April 26 and a more general discussion of the topic was held at the Royal Society on April 30. There now seems to be agreement that the induction of antibody formation to many antigens requires an immunologically specific response to two distinct antigenic determinants by two distinct populations of lymphoid cells. The ultimate precursors of both populations come from the bone marrow, but one population (the T cells) requires, during its maturation, a sojourn in the thymus.

The T cells, said Dr A. J. S. Davies (Chester Beatty Institute), are capable of prolonged re-circulation, they respond *in vitro* to phytohaemagglutinin and populate the "thymus-dependent areas" of the peripheral lymphoid tissues. Although they certainly react with antigen through specific receptors and mediate delayed hypersensitivity and homograft rejection, they are not the cells that go on to secrete the serum antibodies.

T cells further carry on their membranes a specific allo-antigen, θ. Studies with anti-θ serum comprised the bulk of the work described from NIMR. Dr J. Owens described how lymphocytes develop θ antigen during their sojourn in the thymus, and Dr H. H. Wortis said that congenitally thymus deficient mice have no θ positive cells. Mr M. Raff showed that θ antigen is present on all lymphoid cells in the thymus, on 90 per cent of those in the thoracic duct lymph and on a variable proportion of those in the peripheral lymphoid tissues. This proportion is greatly reduced in thymus deprived animals or in those treated with antilymphocyte serum. Dr M. Greaves presented data to show that θ positive cells also carry receptors sharing the Fab determinants but not the Fc determinants of immunoglobulins. For this reason the group at NIMR postulates a distinct immunoglobulin, IgX, on the surface of T cells.

The second population, the cells that make the circulating immunoglobulins, are known as B cells to mark their association with the bone marrow. It became clear that less is known of their life span and recirculation than is the case with T cells. They carry immunoglobulin receptors on their membranes, which are believed to be representative as to class and to affinity of the product that they and their progeny are capable of secreting, but they do not carry the θ antigen.

Cooperation in antibody production has been demonstrated in two separate connexions. One is the formation of antibody to hapten in an animal immunized with hapten carrier complex. In this situation some specific response to carrier determinants is required for anti-hapten antibody to be made. The other is the requirement for both T cells and B cells in order to restore to an irradiated animal its capacity to make antibody to many antigens. Drs K. Rajewsky (Cologne), R. B. Taylor (Bristol) and others showed that it can be accepted that these are two aspects of the same phenomenon. The system devised by Dr N. A. Mitchison (NIMR) for showing this to be so is to transfer, into an irradiated recipient, thymus derived cells from one donor and bone marrow cells from another. The conclusion is that to make antibody optimally to a hapten it requires bone marrow cells primed to the hapten itself and thymus derived (helper) cells primed to the carrier. The elimination of helper cells by treatment with anti-θ and complement, as Raff showed, is further evidence that they are T cells.

Mitchison proposed that cooperation served as a mechanism for concentrating antigen at the B cell receptor, the high local concentration allowing relatively low affinity receptors to be triggered. This model provides an explanation for the observation that the cooperating cell is not needed when high concentrations of antigen are used, or with certain highly polymeric antigens which presumably can react with multiple receptors on a B cell.

The mechanics of cooperation are uncertain. One school of thought, represented by K. Rajewsky and R. B. Taylor (NIMR), believes that T cells and one or more B cells are brought into contact by antigen bound on their receptors. Doubts were expressed by Professors J. L. Gowans (Oxford) and J. Turk (Royal College of Surgeons) as to whether the conditions of cell to cell contact would always be met at sites where the cooperative immune response occurs. As an alternative, it was proposed by Dr P. J. Lachmann (Cambridge) that cooperation was mediated by an antigen-specific "macrophage inhibition factor" secreted by T cells, which binds to local macrophages and there presents antigen to the B cells.

The general validity of the model of cell cooperation that emerged from the two meetings seems to be established and marks an advance in knowledge of the immune response which should influence the planning and the interpretation of experiments throughout immunology. There are some topics—the function of the thymus hormone and antibody formation in humans with immune deficiency states and in chickens after bursectomy or thymectomy—where the implications are so vital that it is a pity that there was no opportunity to discuss them.

ALGAE

Remarkable Membranes

from our Microbiology Correspondent

THE gas vacuoles of the blue-green alga *Microcystis aeruginosa* have been found to have limiting membranes composed entirely of protein, without the lipids usually expected in biological membranes. Gas vacuoles, which are found in the cells of many blue-green algae and some bacteria, seem to regulate the vertical distribution of these organisms in water. Their most remarkable feature now seems to be their limiting membrane, as Jones and Jost have found recently (*Arch. Mikrobiol.*, **70**, 43; 1970).

They lysed algal cells by treatment with penicillin and prepared and purified vacuoles by a combination of liquid polymer partitioning and molecular sieving on 'Sepharose'. In this way the principal soluble and insoluble lysate components such as proteins, pigments, thylakoids and the polysaccharide α-granules were removed. Radioactive cross-contamination tests indicated that the final membrane was only 4 per cent contaminated by extraneous material. The membrane comprised a protein which had 52 per cent non-polar, 18 per cent basic and 10 per cent acidic amino-acids, from which imino and sulphur amino-acids were absent. Isoelectric focusing gave a value of 7 for the isoelectric point of the membrane protein, and its molecular weight was found to be about 14,000.

Membranes from gas vacuoles of *Halobacterium* are composed largely of protein, but allegedly contain nucleic acid and carbohydrate (Stoeckinius and Kunau, *J. Cell Biol.*, **38**, 337; 1968). Jones and Jost's findings, however, suggest that the non-protein components in the bacterial system are contaminants. Nevertheless amino-acid and density characteristics clearly distinguish the membrane protein of *Halobacterium* from that of *Microcystis*.

To complement the chemical data, Jost and Jones have made a structural analysis of the membrane of the gas vacuole of *Microcystis* (*Canad. J. Microbiol.*, **16**, 159; 1970). They found that the vacuole was 70 × 350 × 2·8 nm in size, and composed of a single layer of subunits 2·8 × 4·2 × 3 nm. Frozen-etched preparations show that the inner face of the vacuole has a ribbed appearance and that the ribs are made up from the protein subunits. Each turn of a rib is composed of about seventy subunits and the vacuole as a whole contains about 6,000 subunits. Measurements of folding angles in flattened vacuoles made it possible to determine a value of 25 nm for the distance between subunits in the ribs.

Clearly the membranes of gas vacuoles are very different from other biological membranes in which interactions between lipids and proteins are thought to define the structural and physico-chemical properties. The "unit membrane" concept is not appropriate to the gas vacuole membrane in which interactions are between proteins and proteins. The molecular organization of the membranes of gas vacuole remains to be deciphered, but such protein membranes may be derived from covalent linking analogous to the bacterial peptidoglycan, or non-covalent interaction of the type proposed for virus particle construction. Further investigations of these prokaryotic organelles will certainly be watched with interest.

SYNTHETIC POLYPEPTIDES

Models for Models

from our Molecular Biology Correspondent

ALTHOUGH it is true that the analogy between synthetic polypeptides and proteins, hard pressed in its day, is now barely discernible, these versatile molecules retain a certain fascination, and have become a study in their own right. At the same time the experimental and theoretical treatment of conformational transitions has been based to a great extent on synthetic homopolymers: they have provided the whetstone on which the tools fashioned for the assault on the structure of real proteins are still being sharpened.

The calculation of stable conformations of polypeptide chains is an activity that has so far been judged almost wholly on the basis of its success in predicting the known characteristics of synthetic polypeptides in solution. An interesting example here is the series of esters of poly-L-aspartic acid. Most of these form right-handed α-helices, but others turn out left-handed. Scheraga and his colleagues (Yan *et al.*, *J. Amer. Chem. Soc.*, **92**, 1109; 1970) have performed calculations of the conformations of the three isomeric chlorobenzyl esters (*ortho*, *meta* and *para*) of poly-L-aspartic acid. Their report rouses the confident expectation of a dramatic result—that these polymers, chemically so similar, will differ conformationally—and of the ineluctable convergence of theory and experiment. And so indeed it turns out. In order to improve the physical basis of the calculations, Scheraga and his colleagues have recently introduced molecular orbital methods for determining energy functions. Minimization of energy involves varying all dihedral angles in the residue and calculating a series of energy contributions for each combination. Two large contributions in the aspartates come from the interaction of the amide with the side chain ester dipole, and with the carbon-chlorine bond dipole. In all cases the dipole-dipole interaction energy is lower in the left-handed than in the right-handed conformation, but whereas in the *ortho* and *meta* polychlorobenzylaspartates it dominates the total energy difference between the two states, in the *para* isomer it is insufficient to overcome a torsional energy favouring the right-handed helix. The calculation, it must be said, is delicately poised, the result being a small resultant of large positive and negative terms. Nevertheless, examination of the three isomers (Erenrich, Andreatta and Scheraga, *ibid.*, 1116) bears out the prediction: in four helix-forming solvents the *para* isomer is plainly right-handed, as judged by optical rotatory dispersion, and the other two, with inverted Cotton effects, are left-handed.

Another finely balanced conformational situation occurs in poly-L-lysine and its homologues. Polylysine, as all the world knows, is a random coil in water at neutral pH, but becomes α-helical at high pH, when the side chains are deprotonated. Polyornithine, which has a shorter side chain by one carbon, also undergoes such a transition, but achieves only partial helicity. Hatano and Yoneyama (*ibid.*, 1392) have prepared the next lower member of the series, poly-γ-diaminobutyric acid, and this exhibits scarcely any helix at all at any pH, by optical rotation, circular dichroism and infrared spectrum. Evidently the formation of the polylysine helix depends on the long side chain (a curious result, in that polyalanine, for example, is helical when solubilized in water). When the ϵ-amino groups are blocked with carbobenzoxy functions, the polymers are insoluble in water, but in suitable solvents all are helical, and indeed it is the diaminobutyric acid helix that is most resistant to a helix-breaking solvent. Inter-side chain associations of differing strengths are postulated to account for the results.

A favourite polymer, the object of much experimentation over the years, is poly-L-proline. It has no internal hydrogen bonds, and exists in a left-handed and a right-handed form, the transition between them involving *cis–trans* isomerization about the peptide bond, which is a slow process. Mattice and Mandelkern (*Biochemistry*, **9**, 1049; 1970) have confirmed the surmise that the random coil also exists, and can be elicited by addition of calcium chloride to the aqueous solution. A theoretical analysis of the solvent-dependent transition between the helical forms, I and II, using the lattice formulation of Schwarz, is given by Gaser *et al.* (*Biopolymers*, **9**, 329; 1970) and the agreement with experiment is a telling demonstration of the capacity of modern theory to cope qualitatively and even quantitatively with conformational transitions in homopolymers.

RADIATION PROTECTION

Reducing the Rads

from a Correspondent

THE problem of protecting workers and the public from any harmful effects of ionizing radiation occupied members of the international radiation protection association at their congress in Brighton on May 3–8.

The congress president, Dr W. G. Marley (UKAEA Health and Safety Branch), said that this was an important and continuing task in the application of radiation for the benefit of mankind. The benefits of avoiding future detriment set against the marginal costs of reducing radiation doses led Mr H. J. Dunster (UKAEA Health and Safety Branch) to define a "justifiable dose". If the cost of dose reductions exceeded a few pounds per man rad he considered that the "justifiable dose" would not be much less than the "supportable dose" which was based only on comparison with radiation and other risks. Several other contributors used the man rad cost—the cost of reducing a man's dose by one rad as an aid to decision making between, say, alternative protection schemes or courses of radiotherapy. The International Commission on Radiological Protection (ICRP) fielded a strong team for a round table discussion on the basis and application of their recommendations. Dr E. E. Pochin said that although the accuracy with which risks were known was only approximate, rules and regulations based on ICRP recommendations were precise. Others referred to the biological insignificance of an individual receiving twice the maximum permissible dose, as recommended by ICRP, in one year.

The ICRP listed fruitful areas for research: on the shape of the dose–effect curve at low doses compared with the ICRP linear model and on the risk of taking particles of high specific activity into the body. There was no doubt that ICRP must continue to provide basic guidance on dose limits.

Dr R. H. Mole (MRC Radiobiology Unit) reported that increasing knowledge and understanding showed that none of the working hypotheses is true in more than a rather limited sense. He said that some of the new knowledge came from continuing the follow up of work on well-established groups of irradiated persons, but there was no more evidence than before of measurable effects from radiation exposure of the order of natural background. Dr W. L. Russell (Oak Ridge National Laboratory) reported mechanisms of genetic recovery which ranged from the repopulation of the spermatogonia in the testis to the more direct recovery arising from the repair of mutational and premutational material at the molecular level.

The natural background still attracts considerable attention although situations studied often involve the exposure of only a very few people. Several contributors, notably Dr M Suomela (Institute of Radiation Physics, Helsinki), reported radiation exposure following the ingestion of water rich in radon-222. There is encouragement for studies of the metabolism of plutonium in the commercial prospects of nuclear power from this element. Suggestions for the treatment of plutonium contamination of the lungs included the use of chelating agents in aerosol form as a convenient long term treatment.

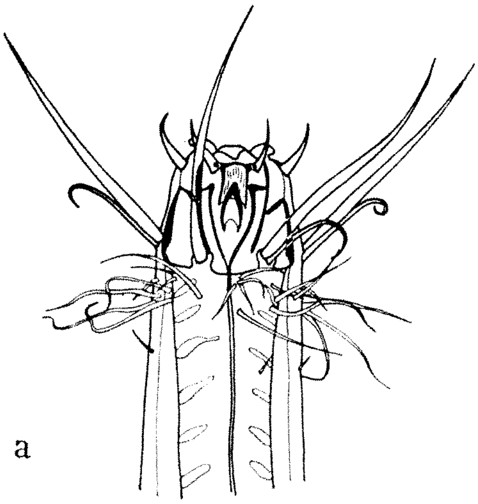
Dr G. M. Obaturov and others from the Ministry of Physics and Power Engineering, USSR, compared the absorbed dose in a human body for neutrons incident from different directions with Snyder and Neufeld's unidirectional data, and concluded that a large correction factor was necessary. A method of applying this correction was suggested by Dr J. Neufeld (Oak Ridge National Laboratory), who proposed a quality index defined as the ratio of the maximum dose equivalent in a phantom (DE$_{\max}$) and a reference dose (D$_{\rm R}$) which could be the absorbed dose in soft tissue in an unperturbed radiation field. The quality index would then be used together with the reference dose to predict dose equivalent.

The potential release of fission products to the atmosphere due to reactor accidents requires constant vigilance in the metabolic pathways to man. Nuclides usually considered to be most important are iodine, strontium, caesium and ruthenium. Most speakers agreed that iodine was likely to be the dominant cause of radiation exposure (unless the reactor system is designed to control iodine release). Dr R. Scott Russell (ARC Letcombe Laboratory) said that in most Western countries the critical pathway is still by way of milk from cows and that strontium-89 is likely to be more significant than strontium-90 in the early stages of a release.

INVERTEBRATES

Discovery in Devon

THESE fearsome little creatures have been discovered between the sand grains of the Exe Estuary in South Devon during an ecological survey of free living nematodes. These two, the male (*a*) and female (*b*) of *Enoplolaimus denticulatus*, belong to one of fourteen new species of nematodes described from the estuary by R. M. Warwick (*Bull. Brit. Mus.* (*Nat. Hist.*) *Zool.*, **19**, No. 4; 1970). On the basis of Warwick's measurements of six specimens of *E. denticulatus*, the heads in the picture are likely to be about 0·05 mm in diameter. Males were on average 3·7 mm long and females 4·3 mm long; this is apparently a relatively large member of the genus.



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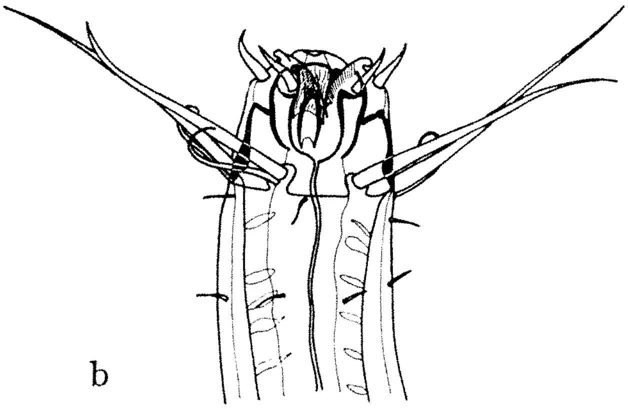

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METABOLIC DISEASES

More Clues from Enzymes

from our Medical Biochemistry Correspondent

NEW methods of diagnosis may be possible for two serious conditions involving inborn errors of complex lipid metabolism. They could stem from the results of enzyme assays of cases of Fabry's and Tay-Sachs diseases.

Fabry's disease seems to result from a lack of galactosyl hydrolase, which acts on the glycosphingolipid galactosyl galactosyl glucosyl ceramide, known as GL-3. Patients with this disease accumulate GL-3 in their plasma, urine and most tissues. The enzyme is found in a particulate fraction in normal spleen small intestine, kidney, brain and liver; it is absent from the mucosa of males with Fabry's disease and present in smaller than normal concentrations in tissues from heterozygous females (this disease is sex linked). Mapes *et al.* (*FEBS Letters*, **7**, 180; 1970) have found that normal human plasma contains at least one enzyme with this activity, whereas the plasma of patients contains no detectable GL-3 galactosyl hydrolase activity.

In the plasma of four normal subjects they found maximum GL-3 galactosyl hydrolase activity at *p*H 5·4 and 7·2, which probably means that two different enzyme molecules catalyse the same reaction. The optimum activity at *p*H 5·4 is similar to that found in the tissues; the four normal subjects all had about sixteen units of activity in their tissues at this *p*H. In the patients there was no activity in the plasma at either *p*H, which means that their plasma could not contain more than 1·5 enzyme units. The activity of GL-3 galactosyl hydrolase in the plasma not only provides an easy method of diagnosis, but also suggests that the condition could be treated. If the normal plasma enzyme activity could be restored artificially the accumulation of glycosphingolipid in the tissues might be prevented.

Tay-Sachs disease is a similar condition which is usually rapidly fatal, and Schneck *et al.* (*Lancet*, i, 582; 1970) have shown that it can easily be diagnosed before birth. They investigated a subject who had given birth to a child who died from Tay-Sachs disease, due to the absence of hexosaminidase A activity—the enzyme hexosaminidase is normally present as fractions A and B. They removed amniotic fluid at fifteen and twenty weeks in a later pregnancy and assayed it and uncultured foetal cells for hexosaminidase A activity, which turned out to be present in trace amounts only. By contrast, uncultured cells from a normal subject contained 28 per cent hexosaminidase in the A form. When the pregnancy was terminated the foetal brain contained 4·5 times as much of the ganglioside GM2 (which is cleaved by hexosaminidase A and B) as a normal foetal brain at fifteen weeks.

The results of the enzyme assays show that it is easy to detect Tay-Sachs disease according to the level of activity of hexosaminidase A at a relatively early stage in pregnancy without the need to culture foetal cells. Schneck *et al.* point out that if further work substantiates their findings effective genetic counselling will be available for the first time for mothers of infants with Tay-Sachs disease.

BRAIN CELLS

Neurones that Count

from our Neurophysiology Correspondent

A TEAM at the University of California claims that the cat's brain contains cells which count. R. F. Thompson K. S. Mayers and C. J. Patterson (*Science*, **168**, 271; 1970) have found that certain cells in the "associative cortex" apparently respond to a particular stimulus in a sequence of stimuli, for example, the fifth or seventh. Thus these cells seem to be coding for number.

The "receptive field", a concept introduced by Adrian in 1929, has become the investigational unit of neural coding. For example, neurones in the cat's visual system can be classified in terms of their responses to light stimuli of varying size, shape, brightness and duration delivered to that part of the retina stimulation of which changes their probability of firing. This is the cell's receptive field, and similar definitions can be made for receptive fields of neurones in other sensory systems. The adequate stimulus for a particular receptive field in the visual system comes from a hierarchy of increasing complexity, from spots of increased brightness or dimness, through edges, bars and slits, to figures containing angles. It is obviously difficult to maintain that the hierarchy will extend to include "gnostic" neurones with receptive fields that need very precise stimulation with complex patterns such as those presented by a mouse, but at least the initial hierarchical stages of pattern recognition seem to have an anatomical basis.

A further elaboration of receptive field is the class of abstraction, for example, recognition of number, which a cell might perform, and so it is interesting that Thompson *et al.* have reported the discovery of neurones sensitive to number in the "association" areas of the cat's cerebral cortex. They recorded the electrical activity of single cells in the brains of anaesthetized cats. The stimuli were sequences of clicks, light flashes or electric shocks, and different neurones responded with an increased probability of firing after different numbered stimuli.

Thompson *et al.* claim that the response is independent of stimulus modality and rate and is repeatably evoked during a long sequence of stimulus presentations. They report the discovery of five counting cells, encoding the numbers 2, 5, 6 (twice) and 7, from an investigated sample of 500 cells. The sequences were of ten stimuli repeated ten times. Within the responses recorded from the cells mentioned the grouping of discharges for particular stimulus number is significantly non-random. But, without a demonstration that such results could not be expected from random sampling of a population of 500 cells, it is better to reserve judgment on the existence of counting cells. It is clear that the cerebral cortex can in some sense count, but there is no *a priori* reason to expect encoding of number to occur at a single-unit level, although it must be expressed as a change in unit activity, though not necessarily in the activity of single, "gnostic", neurones.

HORTICULTURE

Pests of all Sizes

by our Botany Correspondent

GARDENERS who swathe their fruit bushes in string and cotton to protect them from the ravages of birds should be heartened to know that their method has been validated at the East Malling Research Station. The latest annual report from this centre of fruit growing research (price 21*s*) relates how 1 per cent of flowers were injured by house sparrows on blackcurrant bushes surrounded by strands of black thread, while on unprotected bushes 80 per cent of flowers suffered. When harvest time came the protected bushes provided an average of 3·7 berries per truss, compared with 1·1 from unprotected bushes. The respective yields were per bush 222 and 66 grams. A similar scheme is on trial with cherry trees, which have long suffered from hungry birds at East Malling. Trials with trees enclosed in bird proof netting supported on poles are to be extended next year.

During the past year there have as usual been further successes in the continuing battle against viruses. The widely used method for producing material free of viruses by culturing the growing tips of plants treated with heat, to eliminate the viruses, is proving useful with strawberries. Twelve clones freed of viruses in this way have cropped normally in their second year and are now available for release. Several hundred tips of hop have now been set up in culture, but so far no plantlets are ready for transfer to pots. These methods have also been successful with apple, pear, plum and cherry.

Heat has also been used in attempts to rid cherry budwood of overwintering populations of *Pseudomonas morsprunorum*, one of the causes of bacterial canker. Results so far suggest that it is difficult to kill the pathogen without also killing the buds. Treatment with streptomycin, however, seemed to be more promising; after immersion for six hours in 200 or 500 parts per million of the antibiotic, no colonies of the bacteria were detected on treated buds. One of the problems of this work has been identifying *P. morsprunorum* on untreated buds, and so high priority is to be given to improving techniques for isolating the organism.

The microflora of the surfaces of apples are also being identified, in the hope of achieving a better understanding of the way in which non-pathogenic fungi and those able to cause rotting compete for essential nutrients, and also of the changes in the microflora in relation to storage conditions and ripening. So far several yeasts have been identified, including *Torulopsis*, *Candida* and *Rhodotorula*. Fungi present include *Cladosporium*, *Alternaria* and *Phoma*.

CARBON FIBRES

Anisotropic Pores

from our Materials Science Correspondent

Now that it is widely known, in outline at least, how carbon fibre with optimum properties can be manufactured, interest increasingly focuses on the physical properties of the end-product and the way in which the structure correlates with the preparation parameters. For this purpose Ruland in Belgium has been using small-angle scattering of X-rays for several years (*CR Acad. Sci.*, **269**, 1597; 1969). D. J. Johnson and C. N. Tyson at the Textile Physics Laboratory at the University of Leeds have also been applying the technique, and they have recently published some of their findings (*J. Phys. D.*, **3**, 526; 1970).

The angular distribution of small-angle X-ray scattering is particularly sensitive to heterogeneities of electron density and thus to porosity, and it is possible to assess the anisotropy of pore shape. There are alternative theories of interpretation, which presuppose hypotheses about the statistical distributions of pore sizes, but Johnson and Tyson use various internal checks which diminish the arbitrary element in interpretation. They used Rolls-Royce carbon fibres, presumably made from polyacrylonitrile (PAN) and graphitized at different temperatures. Mean pore size and crystallite size and mean surface area of pores were deduced from X-ray scattering measurements as a function of graphitizing temperature and correlated with tensile strength. The latter peaked at 1,250° C, while the pore size suddenly increased at 1,900° C (with, however, no associated discontinuity in measured strength). For the highest graphitizing temperatures, pore size and modulus varied little but strength was not reproducible. The 1,900° C discontinuity is attributed to the plentiful breaking of cross-links, which leaves the structure in a "labile" state.

MOON

Gold and the Glassy Craters

by our Astronomy Correspondent

ONE of the more awkward discoveries of the Apollo 11 mission is that small craters between 20 cm and 1·5 m across, frequently contain lumps of soil coated on their top surfaces with glass, but Professor T. Gold's explanation of this phenomenon in terms of a solar flare powerful enough to cause glazing within the craters has not been immediately accepted (*Science*, **165**, 1345; 1969). The problem is, of course, to invoke glazing within the craters and nowhere else, for there is no evidence that the glazed patches have anything to do with the glass beads which make up such a significant part of the lunar soil. This is why Gold hit upon solar heating as the mechanism, because calculations by D. Buhl, W. J. Welch and D. G. Rea in the *Journal of Geophysical Research* indicate that the temperature within the craters is likely to be 10 per cent or so higher than that of the surrounding lunar surface when the Sun is above the horizon (**73**, 5281; 1968). Thus Gold proposed a solar flare which would have been just powerful enough to start melting the soil within craters but to leave the surface intact.

Obviously people are finding the degree of selectiveness necessary for this process hard to accept, and three papers challenging, or at best expressing doubt, about Gold's hypothesis appear in a recent issue of *Science*. J. Green of the Douglas Advanced Research Laboratories, for example, who shows his hand at the start by admitting his belief that almost all the major surface features are volcanic in origin, finds it easier to believe that volcanic bombs are responsible both for the craters and the glassy patches which they contain (**168**, 608; 1970). Volcanic bombs are large blobs of molten lava protected by a solidified crust which are thrown out of volcanoes. On impact they could produce a crater and their molten contents are likely to freeze rapidly in the lunar environment to leave behind the glassy patches.

Then E. D. Dietz and P. J. Vergano of Owens-Illinois Inc. of Ohio, famous for its glass–ceramic telescope mirrors, point out that the appearance of the glassy patches on the Apollo 11 photographs do not match what might be expected for the glazing of basalt by solar heating (**168**, 609; 1970).

Finally W. R. Greenwood and G. Heiken of the Houston Manned Spacecraft Center suggest that the glass is lunar surface material fused by the impact of the meteorite producing the crater and which has fallen back after the explosion to land on the crater floor (**168**, 610; 1970).

But although Gold agrees that the solar flare hypothesis is not strong, he refuses to accept that the alternatives are any better (**168**, 611; 1970). The drawback to the meteorite hypothesis, for example, is that the explosions are violent enough to make it unlikely that material would fall back precisely to the centres of the craters where the glassy patches are found. Gold also points out a general drawback to theories which have the glass formed at the same time as the craters. Because the glass appears to be barely eroded at all, the implication is that the craters have not been modified since their formation, and this is hard to square with what is known about the size distribution of meteorites.

Thus even Gold would seem to agree that the explanation of the phenomenon is still in doubt. Obviously everyone would like to get their hands on a sample of the glass, but unfortunately this is something that neither Apollo 11 nor 12 was able to provide.

BANDS

Contours from Photons

by our Solid State Physics Correspondent

ONE of the highlights of physical chemistry in the past few years has been the emergence of photoelectron spectroscopy as a means of studying the energy levels in molecules. This has been accompanied by a growing interest in what can be accomplished in solids with photoelectrons, and G. D. Mahan has now suggested that the angular dependence of photoemission should give important clues to the band structure of metals (*Phys. Rev. Lett.*, **24**, 1068; 1970).

Mahan has pursued a calculation for the case of sodium, which seems to be the customary metal on which to test new theories. He shows that electrons excited by a band to band transition in the metal which then leave the solid without being scattered are emitted in a highly anisotropic pattern, which is closely related to the characteristics of the conduction band. The details of photoemission are determined by how a series of cones, dependent on successive reciprocal latticed vectors, is projected outwards through the surface of the material.

For a given frequency of photon, the electrons in sodium have a well defined energy for a particular direction of emission. To calculate the intensity of the electrons, Mahan evaluates the Green's function for the electrons and interprets the resulting intensity distribution in terms of a free electron model. He deduces some typical intensity contours, which must be supplemented by the smooth background from electrons scattered both in the bulk material and by the surface.

To be useful, the technique must be applicable to metals with more sophisticated band structures. Mahan contends that a profitable way of proceeding is to postulate a form of pseudopotential with a number of parameters, calculate the general form of the intensity contours from the pseudopotential, and then set about a least squares fitting operation to give the best value of the parameters from the data. This does not avoid the task of finding the right sort of pseudopotential in the first place, but it does suggest that the scattering data will be quite exacting on the theory, which is the next best thing.

Molecular Markov Processes

by
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Markov processes are encountered in many contexts in physics and chemistry. This review surveys the scope of their application.

THE research worker meeting Markov processes for the first time can be excused a feeling of surprise that such an apparently all embracing concept should have appeared even later in theoretical physics than on the more public side of probability theory. A term which can usefully be applied to subjects as diverse as the weather, the form of a racehorse, note patterns in music or the migration of labour could hardly fail to play as ubiquitous a part in pure science. Yet, although the notion of a Markov process is implicit in some classical studies—for example, the work of Rayleigh[1]—the term has only quite recently entered the physico-chemical vocabulary and is still denied some of the unifying influences it deserves.

When their basic generality is appreciated, a list of common physical Markov processes reads with all the diversity of the examples just given: configuration and growth kinetics of polymer chains, the slowing down of a neutron or hot atom, enzyme reactions, noise in electrical circuits, growth of condensation nuclei, dielectric relaxation. . . .

Their common characteristic is that they can all be represented as the average over an evolution, whether in space or time, of an ensemble of independent sub-systems each of which may undergo change between well defined states with prescribed transition probabilities. Moreover, the latter can be approximated, at least, as being a function only of initial and final states in a transition that is as independent both of the nature of previous steps and the probability of occupation of the states themselves. This restricted dependence on past history, which is the essence of the Markov process, often arises quite naturally in a physical model. For example, a molecule in a dilute gas "forgets" its past dynamics during the mean path between collisions and on next colliding scatters with probabilities determined only by its present velocity; the orientation of links in a polymer chain may be made independent of previous configurations by restricting the excluded-volume effect, and so on.

Mathematics of Markov Processes

Mathematically, the effect of the restrictions is that the transport equations for the evolution of probabilities in the space of states are reduced to linear form in which their solution is usually possible, at least by numerical methods. With this built-in loss of memory the Markov process might be described as "partially deterministic", occupying a position somewhere between the pure random process at one extreme and the pure deterministic process at the other. But the loss of memory is a subtle effect and it is important not to confuse the abrupt cut-off in dependence on the past implied in the transition probabilities with the slower-diminishing autocorrelation in the passage between successive states, which can extend over many transitions.

The mathematics of Markov processes is not as a rule exceptionally difficult but suffers considerably from complications of notation. Because my chief intention is to survey the scope of applications rather than give a detailed exposition, it will be convenient to express the working equations somewhat symbolically, as, for example, in a brief outline of quantum mechanics. Further detail of the mathematical background is readily accessible in several excellent monographs[2-4].

The general Markov process can be represented by the following equation, whose terms must be interpreted somewhat differently according to the case in question

$$\dot{\mathbf{P}}(t) = \mathcal{A}\,\mathbf{P}(t) \tag{1}$$

The left hand side gives the rate of change of the populations of states and this is equated to the effect of a linear operator $\mathcal{A}$ acting on the populations themselves. I shall usually refer to the independent variable t as time, though it may equally well be a spatial parameter such as an index numbering the links in a polymer chain. If the states are discrete (for example, quantum levels, steps on a lattice) $\mathbf{P}(t)$ is a vector

$$\{p_1(t),\ p_2(t),\ \ldots\ p_k(t)\ \ldots\}$$

possibly infinite, the components of which list the probabilities $p_k(t)$ for a sub-system of the ensemble to be in state k at time t. If the process is one in which t varies continuously $\dot{\mathbf{P}}(t)$ is to be taken as the derivative $\mathrm{d}\mathbf{P}/\mathrm{d}t$; if, on the other hand, t varies in fixed intervals it will represent a difference $\Delta\mathbf{P}(t) = \mathbf{P}(t+1) - \mathbf{P}(t)$. The states may, however, be continuously distributed (for example, orientations of links in a freely jointed chain, energy-states of a classical oscillator) and $\mathbf{P}$ then represents an ordinary probability distribution function $P(x,t)$ of the continuous state variable and time.

The nature of the operator $\mathcal{A}$ depends very much on the type of states involved. If these are discrete $\mathcal{A}$ is a matrix the elements of which are related to the transition probabilities K_{ji} for passage between states i and j. (Actually $A_{ji} = K_{ji} - \Sigma_j K_{ji}$.) If the states are continuous $\mathcal{A}$ is an integral operator based on a transition kernel $K(x,y)$ measuring the probability that a system in state x will make a transition to a state in a neighbourhood dy about y in unit time. The dimension of A_{ji} or the range of the kernel K may be finite or infinite as appropriate.

Initial-value Problems

The usual object of calculations on a Markov process is to obtain a full solution of the initial-value problem, that is, expressions for $\mathbf{P}(t)$ at all t subject to some initial distribution $\mathbf{P}(0)$. Sometimes less information will suffice, such as the time-dependence of some average quantity governed by $\mathbf{P}(t)$ or in some cases simply the equilibrium distribution ($\mathbf{P}(\infty)$ to which a given system may tend after

Table 1. SOME ATOMIC AND MOLECULAR MARKOV PROCESSES

Process	States	Linearity assumption	Type*	Desired result	Peculiarities	Reference
Vibrational relaxation	Quantum levels	Dilute system in heat bath	T2 or 4	Relaxation times		5
Vibrational dissociation	,, ,,	,, ,,	T2 or 4	Reaction rate	Passage-time problem	5
Polymer configurations	Orientation of chain links	Short-range excluded vol.	S1 or 3	End-to-end length distrib.		9, 10
Small-system kinetics	No. of molecules reacted	Implicit	T2	Reaction probabilities	Pure death process	12–15
Nucleation kinetics	N-aggregates	Stepwise growth	T2	Growth rate and time-lag	Birth and death process	16–19
Brownian motion	Position or velocity	Dilute system in heat-bath	T4	Evolution, autocorrelation	Gaussian Markov. Classic literature	33–35
Thermalization kinetics	Energy of atom or neutron	,, ,,	T4	Relaxation times. Reaction rates	Continuous spectrum	20
Spin systems	Spin states	Dilute spins in lattice	T2	Response to applied field	Usually 2-state process	44

* I use the following shorthand notation for the four types of spatial (S) and temporal (T) processes. (1) Discrete states/discrete time (or position); (2) discrete states/continuous time; (3) continuous states/discrete time: (4) continuous states/continuous time.

long times. In this, of course, all memory of the initial conditions $\mathbf{P}(0)$ is erased.

In a casual approach to the problem it will be clear that some progress can be made simply by carrying out a systematic "book-keeping" procedure for the flow of sub-systems as time evolves. Symbolically this is equivalent to carrying out the continuous-time solution of equation (1) in the following form

$$\mathbf{P}(t) = \exp(\mathcal{A}t)\mathbf{P}(0) = \{1 + \mathcal{A}t + \tfrac{1}{2}\mathcal{A}^2t^2 \ldots\}\mathbf{P}(0) \qquad (2)$$

Here the powers of the operator $\mathcal{A}^n$ are to be interpreted simply as the operation repeated n times. Although this is a practicable procedure at short times, it rapidly becomes complicated as time increases and is unlikely to give much information on the behaviour of the system as t approaches infinity.

Indeed, if computations were limited to the book-keeping approach, very little of any generality could be said. But the usefulness of the theory of Markov processes as developed during the past 30 years lies in the recognition that the powers of the operator $\mathcal{A}$ or, more straightforwardly, the solution of equation (1), can be expressed in terms of the eigenvalue properties of the simple operator. Thus, if $\mathcal{A}$ has an eigenvalue λ_k and an eigenvector (or eigenfunction) φ_k, that is

$$\mathcal{A}\,\varphi_k = \lambda_k\varphi_k \qquad (3)$$

it is easy to see that a term $\varphi_k \exp(\lambda_k t)$ will be a particular solution of equation (1). If the whole spectrum of λ_k and φ_k for the operator can be determined, then it is usually possible to construct a general solution appropriate to the given initial conditions by a weighted superposition of such terms. I shall illustrate this in later examples.

Several crucial properties follow from the eigenvalue form of the solution. First, it is obvious that, for probabilities to be conserved with increasing time, the operator $\mathcal{A}$ must have eigenvalues λ_k which are all negative or at most zero. ($\mathcal{A}$ is negative semi-definite.) Furthermore, if there are physical requirements that the system should tend to equilibrium as time approaches infinity, then there must be some distribution $\mathbf{P}(\infty)$ with the property that $\dot{\mathbf{P}}(\infty) = \mathcal{A}\mathbf{P}(\infty) = 0$. That is to say, a well formulated operator $\mathcal{A}$ for this case must possess at least one zero eigenvalue $\lambda_0 = 0$ which has the equilibrium distribution $\mathbf{P}(\infty)$ as its corresponding eigenvector. A further simplifying property possessed by most physical systems is the detailed balance condition. This makes it necessary for the flux between any pair of states in equilibrium to be balanced, that is, for discrete states: $p_i(\infty)K_{ji} = p_j(\infty)K_{ij}$. Mathematically, the effect of this is to impose a symmetry property on the operator $\mathcal{A}$ with the very important consequence that all its eigenvalues must be real numbers, as I have anticipated earlier in this article. Thus the oscillatory behaviour associated with the occurrence of complex eigenvalues in systems of equations like (1) is ruled out in most physical examples.

With the Markov process recognized as an eigenvalue problem, many of the properties and techniques familiar in quantum mechanics and the theory of vibrations can be expected to find an application. Indeed, there is considerable scope for perturbation theory, variational calculations, the WKB method, the exploitation of symmetry in relation to the degeneracy of eigenvalues, and so on. Some of these possibilities will be evident in the rest of this article, where I shall attempt to classify both the types of Markov process which arise in physics and chemistry and the particular questions of interest in their solution. The list of processes in Table 1 is by no means comprehensive but should serve to focus the variety of contrasting objectives and assumptions which are possible within the general framework of Markovian theory. I shall only consider the more topically interesting cases in detail.

Vibrational Relaxation and Dissociation

If a collection of diatomic molecules is dispersed in some foreign heat-bath gas with which it is not in thermal equilibrium, transitions between the vibrational energy levels may occur and the evolution of the whole system can be treated as Markovian so long as (*a*) the partial pressure of oscillators is low enough that only collisions with heat bath molecules need be considered and (*b*) the whole gas is sufficiently dilute that there are no dynamical correlations between successive collisions. The transition probabilities K_{ji} for changes of vibrational state must be assigned, for example, by the Landau–Teller model, and will, of course, be a function of heat bath temperature[5,6] (Fig. 1).

These assumptions lead to a transport equation of the following form, in which the constant ω multiplying the second term is simply the oscillator–heat-bath collision number.

$$\mathrm{d}p_i/\mathrm{d}t = \Sigma_j K_{ij} p_j(t) - \omega\, p_i(t) \qquad (4)$$

(that is in matrix terminology: $\mathbf{A} = \mathbf{K} - \mathbf{I}\omega$). The solution for $p_i(t)$, the proportion of oscillators in level i at time t, can then be written

$$p_i(t) = \Sigma_k \alpha_k \varphi_i^{(k)} \exp(\lambda_k t) \qquad (5)$$

where the constants a_k are to be fitted according to the initial distribution $p_i(0)$. The existence of one zero

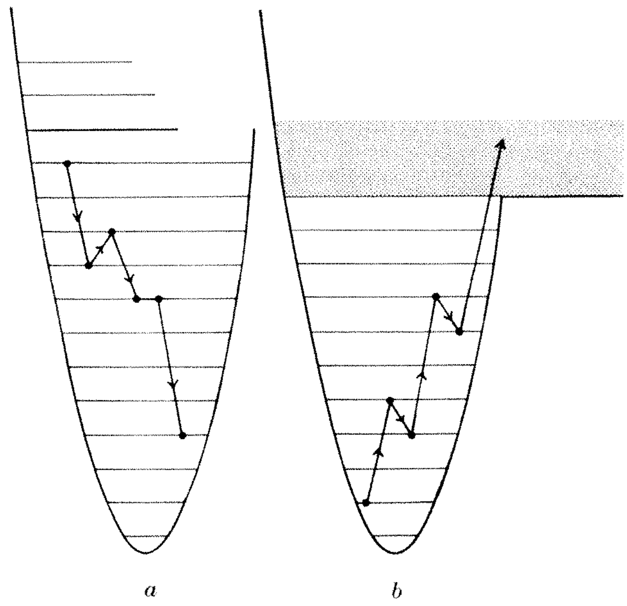

Fig. 1. Markov processes on the vibrational states of a harmonic oscillator. *a*, Simple relaxation; *b*, dissociation at an absorbing barrier.

eigenvalue $\lambda_0 = 0$ guarantees evolution to the equilibrium Boltzmann distribution $p_i(\infty) = \varphi_i^0$. Certain difficulties arise from the essentially infinite character of the set of states, but the eigenvalue analysis can sometimes be carried out in exact form even for the infinite set[5,6].

In the dissociation problem the set of states, and correspondingly the matrix K_{ji}, is truncated at some critical level i^* at which oscillators are supposed to dissociate and become removed from the system. (Statistically i^* represents an absorbing barrier.) Physically it is clear that in time all oscillators will become dissociated (that is, $p_i(\infty) = 0$ for all i); mathematically this comes about because the smallest eigenvalue is perturbed so that now $\lambda_0 \neq 0$. One can attempt to compute, in increasing order of difficulty: (*a*) the mean time taken for oscillators to reach level i^* (the mean first-passage time to reaction); (*b*) the time-dependent concentration $C(t)$ of all unreacted oscillators, and (*c*) the actual time-dependent distribution function $p_i(t)$ for all levels.

If the dissociation limit i^* is relatively high or the temperature of the heat bath relatively low, the absorbing barrier may be treated as a small perturbation on the infinite transition probability matrix K_{ji}. The following results are then obtained. (i) All transients other than $\exp(\lambda_0 t)$ decay relatively fast, leaving an effective Boltzmann distribution throughout the reaction. (ii) The total unreacted population decays by $dC(t)/dt = \lambda_0 C(t)$, that is, the smallest eigenvalue $|\lambda_0|$ becomes the ordinary unimolecular rate-constant. (iii) The mean first-passage time to reaction becomes equal to $-1/\lambda_0$. (iv) The Arrhenius law for the effect of barrier-height and temperature on rate of reaction applies[5,6].

Although attempts have been made to apply a similar scheme to the theory of unimolecular reaction of polyatomic molecules, the difficulty of specifying both the transition probabilities and the states themselves has prevented any very effective solutions being obtained[7,8].

Random Walks and Polymer Configuration

The ordinary random walk in which a jointed chain has its links directed arbitrarily in free space or on a lattice is the simplest model for obtaining the approximate statistics of end-to-end length distribution in a polymer chain. If a set of possible states is taken as the orientations of the *i*th link, the process, as described, is purely random. If, however, it is ruled that a link cannot be reversed along the previous step or intersect an earlier joint, then a Markovian element is introduced with certain transition probabilities becoming zero. In practice it is difficult to allow for interactions further than about three steps back in the chain, but this is sufficient to give some account of the short range contribution to the "excluded-volume" effect (Fig. 2). The eigenvalue analysis of Markov processes on a lattice can be carried out and, as to be expected, there are degeneracies resulting from the symmetries present. Mean end-to-end distances are significantly longer than for the purely random case, though the model is still very far from representing the configuration of a real polymer molecule[9-11]. The problem is not merely one of complexity; some serious conceptual weaknesses in the usual Markovian approach have recently been pointed out[10].

Small System Kinetics

When very few molecules are involved in a chemical reaction, the normal, deterministic rate equations of chemical kinetics cannot apply, fluctuations occur and one can only ask for a probability that, after time t, some number i of an initial total of N molecules remains unreacted. This situation can be treated as a Markov process on N states in which the state i simply corresponds to "i molecules unreacted". For irreversible reactions the behaviour will be that of a "pure death" process with transition probabilities which are zero except in the few cases corresponding to reaction. Thus for the stochastic bimolecular reaction $2A \rightarrow B$ the only finite transition probabilities are of the form $K_{i-2,i} \propto i(i-1)$. A considerable number of articles have given the analysis of such systems[12-15]. In favourable cases the complete distribution function $\{p_k(t)\}$ for the probability of k molecules left unreacted after time t can be obtained; in others various approximations can be made leading to the time-dependence of the moments of this. Usually the mean amount of reaction at time t satisfies a normal, deterministic kinetic equation, but in a stochastic system the law of mass action is only approximately valid[14].

Reactions to which a stochastic approach has been applied include the following: $A \rightarrow B$, $2A \rightarrow B$, $A \rightarrow B \rightarrow C$, $2A \rightarrow B \rightarrow C$, $2A \rightleftharpoons B$ and $E + S \rightarrow C \rightarrow E + P$. The latter will be recognized as the Michaelis–Menten scheme for enzyme kinetics[15]. Because, on general statistical-mechanical grounds, the fluctuations from deterministic behaviour can be expected to disappear as $N^{-\frac{1}{2}}$, it is hardly surprising that no direct measurements of chemical

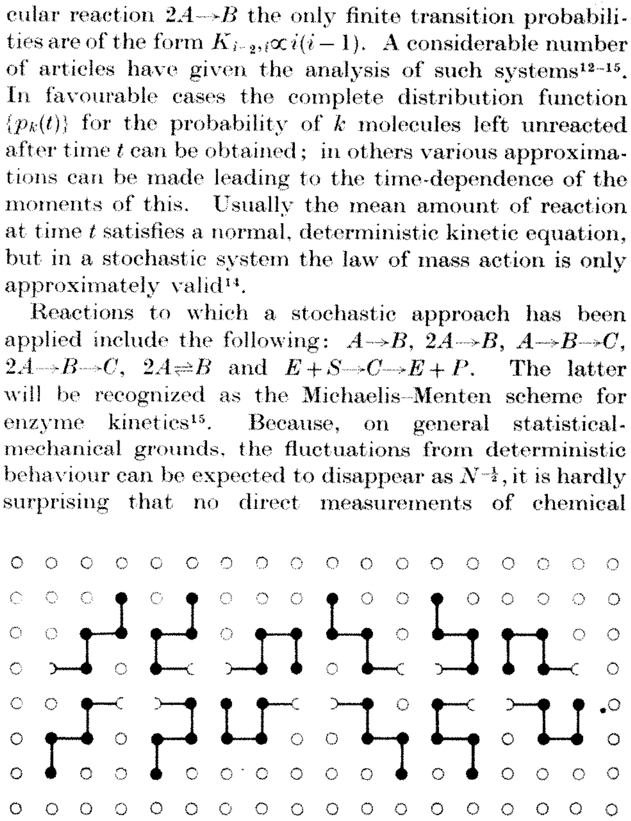

Fig. 2. Markov lattice model for a two-dimensional polymer chain. The twelve basic "states" shown can be combined to generate configurations with short range excluded volume. About half the possible combinations overlap and are given zero transition probability; the rest may be assigned consistent K_{ji} values. (After Montroll[11].)

kinetic fluctuations seem to have been made. (An obviously exceptional case would be the first order decay of a very few atoms of some short lived synthetic radioisotope.) If stochastic effects are ever important in kinetic systems it seems likely that they will be detectable only in specially sensitive situations; for example, the induction period to an explosion, or reactions in certain living systems. Most experimenters will continue to suspect extrinsic errors when they obtain variable kinetic results in several runs.

Nucleation and Droplet Growth

There is good reason to believe that the growth and evaporation of small droplets in a supercooled vapour proceeds by addition or loss of one molecule at a time to the surface of the growing nucleus[16-19]. This makes it possible to treat the kinetics of nucleation as a classic "birth and death" Markov process in which the states are indexed by the number of molecules in the drop and transitions are only allowed between states i and $i \pm 1$. The transition probability matrix is then of tridiagonal form and the equations (1) reduce to a set of differential difference equations. The situation in the growth kinetics of certain polymers such as rubber latex is similar except that the reaction is usually irreversible and the process is of the "pure birth" type with constant growth probabilities[19]. In both cases a "source-term" is introduced for the addition of monomer at a constant rate and in the nucleation problem an absorbing barrier is usually introduced at some critical size i^*. Assuming reasonable models for the pattern of transition probabilities, it is then possible to calculate both the steady state flux through the states and the time-lag in setting this up. In the case of polymerization it is the distribution of chain lengths after time t which is of principal interest because this, unlike the size of nucleating droplets, is an experimentally measurable quantity.

Thermalization Kinetics

Of all the physical Markov processes discussed here the most interesting and mathematically complex is probably that which governs the cooling of a hot atom or subatomic particle in a homogeneous heat bath. A vast literature exists on the subject of neutron thermalization[20] and some of the ideas originating in the field have more recently been applied to the kinetics of "hot atom" reactions and other rate processes in chemical physics[21].

The problem usually considered is to obtain the time-dependent energy distribution, $P(\varepsilon,t)$, of a set of translationally "hot" particles which lose energy by collisions with the moderator. In chemical kinetics the interesting particle is often a hydrogen atom produced by photolysis or electrical discharge, or alternatively a recoil tritium atom. The thermalization process can be taken to be in competition with various possibilities for absorption and chemical reaction.

Under assumptions similar to those described for oscillators, the system can be treated as Markovian and governed by a scattering kernel $K(\varepsilon,\varepsilon')$. This function is known explicitly for a few simple cases such as the hard sphere model[22] and is, in general, a sensitive function of the temperature of the heat bath and the mass ratio for the system and heat bath particles. The transport equation which must be solved is then of the form

$$\frac{\partial P(\varepsilon,t)}{\partial t} = \int_0^\infty K(\varepsilon',\varepsilon)\ P(\varepsilon',t)\,\mathrm{d}\varepsilon' - Z(\varepsilon)\ P(\varepsilon,t) \qquad (6)$$

Although at first sight similar to the continuous form of equation (4), its mathematical properties are radically different on account of the energy dependence in the collision number $Z(\varepsilon)$. This can be shown to give rise to a continuum region in the spectrum of eigenvalues which begins at the point $\lambda^* = Z(0)$ and extends over the whole region where $Z(\varepsilon_\lambda)$ can be equal to λ for some ε_λ. In addition there is usually an infinite set of discrete eigenvalues with a point of accumulation at the continuum threshold λ^*. For the hard sphere case the collision number $Z(\varepsilon)$ increases monotonically from $Z(0)$ so that the spectrum is, as shown in Fig. 3b, a form reminiscent of the hydrogen-atom spectrum in quantum mechanics[23,24]. Several[25-28] quite original mathematical techniques have been devised for the computation of both continuous and discrete parts of the spectrum, but these are very difficult to apply, and it is probably true to say that the physical significance of the continuum eigenfunctions, which are (Schwartz) distributions, is still insufficiently understood.

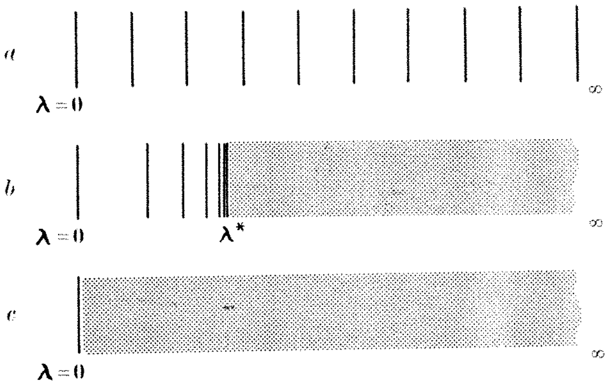

Fig. 3. Eigenvalue spectrum for the thermalization of a test-particle with hard-core interaction. a, The Rayleigh gas. Mass-ratio $\gamma \to 0$. b, The uniform gas ($\gamma = 1$). c, The Lorentz gas ($\gamma \to \infty$). (Schematic only.)

Nevertheless, certain properties of the solutions can be recognized. For example, if the mass of the heat bath particle decreases towards zero, the temperature remaining constant, the zero velocity collision number, for hard interactions at least, must tend to infinity so that the whole eigenvalue spectrum should become discrete in this limit. The resulting spectrum and its corresponding initial value solution $P(\varepsilon,t)$ is then the three-dimensional analogue of Rayleigh's classical exact solution for the one-dimensional gas of infinitely heavy particles; a special case of Brownian motion[29,34]. At the other extreme, when the mass of the system particle becomes very small, $Z(0) \to 0$ and the whole problem becomes dominated by the continuum. This is the case of the "Lorentz gas" undergoing "inverse Brownian motion". It is not surprising that this process is much less well understood than the "Rayleigh gas".

Diffusion Processes

When a Markovian system is dominated by transitions between "close" continuous states or nearest neighbour discrete ones, its mathematics has much in common with that of a diffusion process in ordinary space. With this very considerable restriction, equations of the form (4) and (6) can be approximated by a partial differential equation in a continuous variable and time, which takes the following form[31-34]

$$\partial P(x,t)/\partial t = (\partial/\partial x)\{\alpha_1(x)P(x,t) + \alpha_2(x)\partial P(x,t)/(\partial x)\} \qquad (7)$$

This "Fokker–Planck" equation can be thought of as a generalized diffusion equation with a built-in drift to equilibrium and a state-dependent diffusion constant. The functions $\alpha_1(x)$ and $\alpha_2(x)$ can be derived by systematic procedures from the transition kernel or, in some cases, by a smoothing approximation applied to the tridiagonal matrix for the birth-and-death process. Formally the success of the equation depends on the validity of expressing the integral operator $\mathscr{J}$ as a differential operator of infinite order, and then throwing away all but first and second-order terms—a procedure involving more sleight of hand than mathematical rigour.

A system the evolution of which is described by equation (7) is said to undergo a Gaussian Markov process and has the important property that the "autocorrelation" between successive states decays exponentially[34]. Although in physics the Fokker–Planck equation is usually thought of in the context of Brownian motion, it is also a useful approximation in the treatment of several of the processes already mentioned—nucleation, polymerization and thermalization in the Rayleigh and Lorentz limits. Other applications worth mentioning are the Kramers theory of unimolecular reactions as a Brownian motion in phase space[37], and the "Alkemade diode" problem where it describes the fluctuation of charge in a stochastic R–C circuit[38].

There are, however, serious pitfalls in the use of diffusion approximations. The Fokker–Planck equation will always tend to falsify the higher eigenvalues of the spectrum and can give no account of any continuum region; this must always lead to significant errors at short times and with sharply peaked distributions. It has recently been possible to demonstrate these discrepancies and map out the region of validity of the F–P equation for the hard sphere gas by comparing eigenvalue spectra obtained from the differential and integral equations[39].

Deeper Role of Markofficity

In summarizing these physical realizations of the Markov process I have concentrated on exposing their variety at the expense of mathematical subtleties. Rather deliberately I have avoided emphasis on Brownian motion and the Gaussian Markov process which has so dominated the statistical physics literature in past decades. Though the importance of the Brownian motion process must not be underestimated, the tendency to treat it as a prototype for all physical stochastic processes has been to some extent unfortunate, the result, it would seem, of a long standing reluctance to face the complexities of an integral equation so long as the problem could be forced into a more familiar, if misleading, differential form. The breaking of this habit, within the physical sciences, is largely to the credit of the nuclear reactor theorist whose special Markov processes are of a practical importance that need hardly be emphasized. When fully translated from the rather peculiar language of the subject, the mathematical fall-out from applied neutron scattering and transport theory may prove to be one of the most impressive examples of pure science being led by technology.

The cases I have considered are all rather specific physical or chemical systems; it would be wrong not to mention the deeper aspects of the Markovian property which seem to lie close to the foundations of statistical mechanics and quantum theory and are perhaps inseparable from the idea of irreversibility in nature. It seems widely accepted that the paradoxes of time-reversal asymmetry may be described, if not actually explained, in terms of a Markovian "forgetting" of past history in a large system, though it is not clear how this comes about or whether it must necessarily have a quantum mechanical origin. The Kac–Ehrenfest "dog-flea" model of an irreversible process is a Markovian thought experiment compellingly suggestive of some central role[41,42].

At the same time considerable efforts are in hand to connect the non-deterministic aspects of quantum equations of motion with some kind of Markovian or more general stochastic process[43]. A certain difference of opinion seems to exist as to whether such a reconciliation, even if possible, would be of deep significance, or merely a superficial parallel. If the former view should come to be justified, the Markov process might conceivably attain a central importance in the scheme of natural laws which would dwarf altogether the special applications considered here.

The citations in the reference list are not intended to give a full account of work on the relevant topic or necessarily to indicate priority. Where possible I have quoted an early, key paper together with a more recent one containing a useful bibliography.

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Gene Order of RNA Bacteriophage M12

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In vitro translation of M12 RNA nascent strands, isolated from the M12 replicative intermediate, revealed the gene order: 5′–A-protein–coat protein–replicase–3′.

THE genomes of RNA phages serve both as templates for phage specific RNA replication and as messengers for protein synthesis. These genomes (mol. wt. $1\cdot1\times10^6$) contain three complementation groups which code for the three gene products: coat protein, maturation (A) protein and RNA replicase[1–4]. During phage reproduction, coat protein is made in much greater quantities than the other two phage proteins[5]. This led to the suggestion that the coat protein gene might be located nearest to the 5′ end of the phage RNA molecule, a hypothesis derived from polarity studies carried out with polycistronic bacterial messengers. Initial results were consistent with this idea and, furthermore, suggested that the replicase gene was nearest to the 3′ end of the molecule[6–8].

We present here the complete gene order of RNA bacteriophage M12, obtained by studying the translation of nascent RNA strands *in vitro*. The replication of phage RNA occurs linearly from the 5′ to the 3′ end of the molecule. As the nascent strands grow, they should first be translated into the protein whose gene is nearest to the 5′ end. Larger strands should then contain information for the second and, finally, for the third phage specific protein.

Six fractions of nascent strands, designated A to F, were isolated from the replicative intermediate of the RNA bacteriophage M12 and purified according to their molecular weights (Table 1). By using these nascent strands as *in vitro* messengers we have established the following gene order: 5′–A-protein–coat protein–replicase–3′.

Translation of Nascent M12 RNA Strands

Three criteria were used to identify the protein products synthesized in an *Escherichia coli in vitro* system under the direction of nascent strands: coelectrophoresis of the products on polyacrylamide gels with the phage specific proteins synthesized both *in vivo* and *in vitro* under the direction of phage M12 RNA (Figs. 1*i* and *k*); the absence of histidine from the phage coat protein[13] (Fig. 1*k*); and the repression of replicase synthesis but not of coat protein synthesis by native coat protein[6,7,14–17] (Fig. 1*k*).

Using the nascent M12 strands as messengers, the shortest RNA fragment able to direct protein synthesis was fraction C (9S) (Figs. 1*a* and 1*b*). Apparently, nascent strands A and B (1·4S and 3·5S respectively) are too small to stimulate the synthesis of a trichloroacetic acid precipitable product. Although nascent strand C was primarily translated into many unidentifiable short peptides, it did direct the formation of some larger protein material. This larger material cannot be coat protein, for it has a higher molecular weight and contains histidine. It must therefore be a fragment of incomplete replicase or A-protein.

Nascent strand D (14S) stimulates the synthesis of larger peptides than does nascent strand C, some of which migrate in the region of the A-protein (Figs. 1*c* and 1*d*). No distinct A-protein or replicase peak can be detected, however. Some protein material which migrates like coat protein can also be observed. Two facts, however, indicate that this material cannot be coat protein: it has a high, constant histidine : leucine ratio, whereas coat protein does not contain histidine; and its synthesis can be repressed by M12 coat protein (Fig. 1*c*).

Nascent strand E (19S) is the shortest RNA fraction to stimulate the synthesis of a clear coat protein peak, identified by its gel position and its lack of histidine (Figs. 1*e* and 1*f*). Some of the histidine containing material made from this strand has a higher molecular weight than that found under the direction of nascent strand D, but still only a minute peak can be observed in the region of the replicase protein. Beginning with nascent strand F (25S), a sharp histidine containing peak can first be observed in the position of the replicase (Figs. 1*g* and 1*h*). The ratios of replicase to coat protein synthesized under the direction of nascent strand E, strand F and phage M12 RNA increase dramatically with the size of the RNA (Table 2). These results show that the coat protein gene immediately precedes the gene for M12 replicase, which agrees with results obtained from sequence studies carried out with phage R17[24,25]. Furthermore, because the informational content of strand F is almost as great as that of intact phage RNA, the replicase gene must be localized very near to the 3′ end. Thus the histidine containing material appearing before the coat protein (nascent strands C and D in Figs. 1*a*–*d*) must have been

Table 1. PHYSICAL PROPERTIES OF NASCENT STRANDS ISOLATED FROM THE REPLICATIVE INTERMEDIATE OF BACTERIOPHAGE M12

Nascent RNA strand	Sedimentation value	Approximate mol. wt.	Average informational content (amino-acids)
A	1·4S	5×10^3	5
B	3·5S	$2\cdot5\times10^4$	25
C	9S	$1\cdot5\times10^5$	150
D	14S	$3\cdot3\times10^5$	330
E	19S	$5\cdot7\times10^5$	570
F	25S	$9\cdot5\times10^5$	950
Phage M12 RNA	27S	$1\cdot1\times10^6$	1,100

The replicative intermediate (RI) of M12 RNA was isolated as described by Francke and Hofschneider[9]. The nascent strands were obtained from the RI by heat denaturation and thereafter were separated by zonal centrifugation ('B XIV' rotor, 47,000 r.p.m., 4 h, 20° C) in a 10–30 per cent sucrose gradient in 0·1 M NaCl, 0·05 M Tris-HCl (*p*H 6·9), 0·001 M EDTA, 0·1 per cent sodium dodecyl sulphate (SDS). Six fractions of nascent RNA strands were obtained and further purified by conventional band sedimentation. Finally, the peak fractions were concentrated by ethanol precipitation. The sedimentation constant of nascent strand D was determined by co-sedimenting a sample with infectious M12 RNA. The sedimentation constants of the fractions A, B, C, E and F were calculated[12] from this value. The approximate molecular weights of these nascent RNA strands, relative to that of complete phage RNA, were determined by the method of Studier for single-stranded DNA[10]. Further details will be given elsewhere. No random fragmentation of RNA nascent strands could be detected throughout the preparation procedure. The specific infectivity (p.f.u./$A_{260\text{ nm}}$) of the purified and denatured RI was 0·18 with respect to single-stranded plus strands. This value is close to the theoretical value of 0·5 for completely double-stranded replicative form phage RNA. The specific infectivity of the nascent strand material remained practically unchanged throughout the subsequent purification steps. When the $A_{260\text{ nm}}$ values of the six fractions of nascent strands after zonal centrifugation were plotted as a function of their molecular weights as given in Table 1, either before or after the final band sedimentation, a linear correlation between $A_{260\text{ nm}}$ and molecular weight was found for fractions A to E. The average length of the nascent strands was determined as 0·3 of the complete phage RNA. This value agrees with that described by Robertson and Zinder[11], who checked the integrity of their material by another method. In accordance with their result, the molar quantity of fraction F was less than that found for the other nascent strand fractions.

Table 2. RATIOS OF M12 REPLICASE/COAT PROTEIN SYNTHESIZED *in vitro* UNDER DIRECTION OF NASCENT M12 RNA STRANDS

Nascent RNA strand	Replicase/coat protein
E	$< 10^{-2}$
F	6×10^{-2}
Phage M12 RNA	6×10^{-1}

Ratios were determined from the total radioactivity (c.p.m.) of ^{3}H-leucine incorporated into M12 replicase or coat protein, respectively, as measured from the polyacrylamide gel patterns shown in Figs. 1*e*, *g* and *i*.

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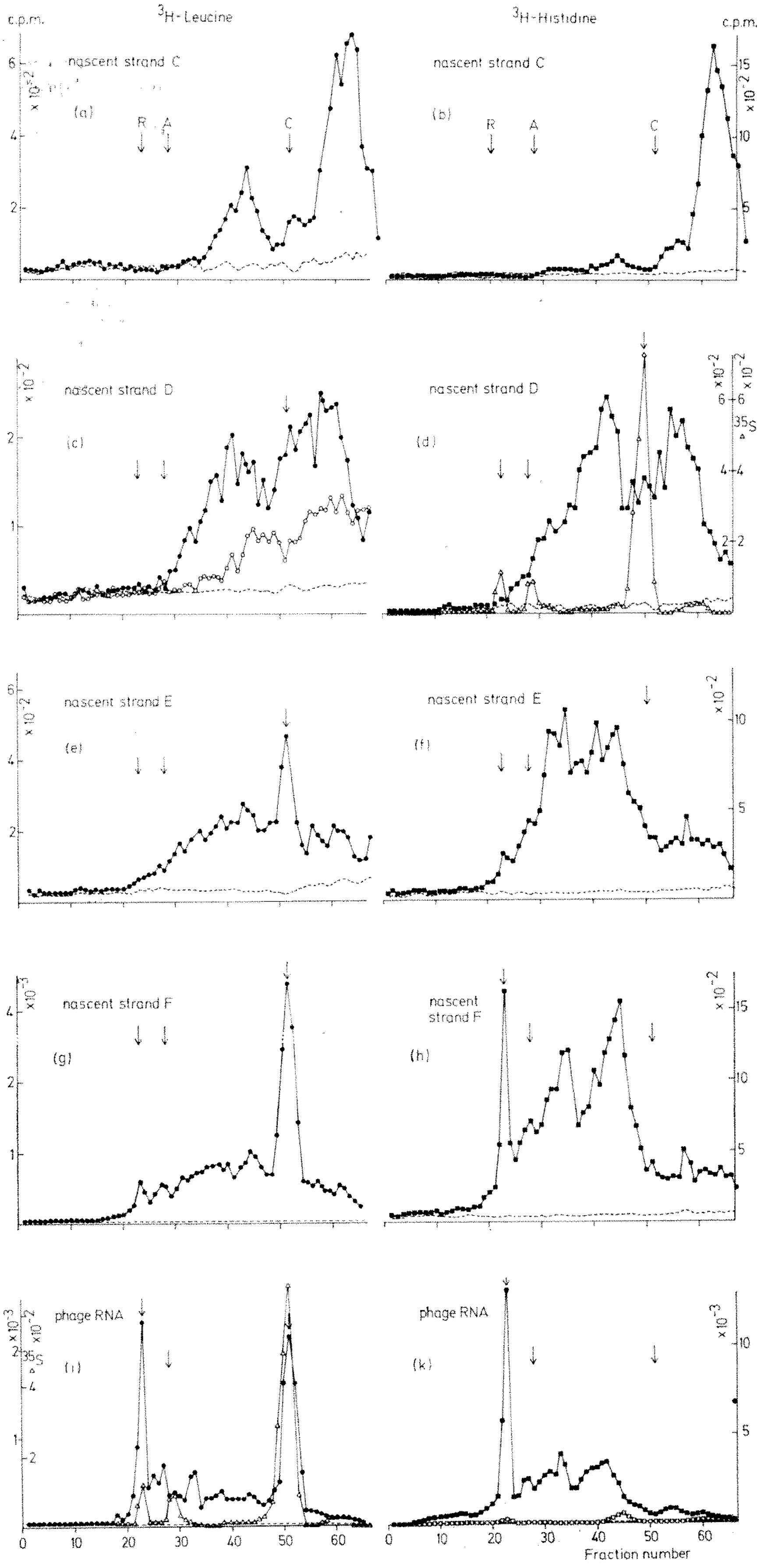

Fig. 1. Polyacrylamide gel electrophoresis of proteins in the cell-free extract of *E. coli* in the presence or absence of nascent M12 RNA strands. ●—●, ^{3}H-leucine labelled proteins synthesized under direction of nascent M12 RNA strands (*a*), (*c*), (*e*), (*g*) and phage M12 RNA (*i*). ■—■, ^{3}H-histidine labelled proteins synthesized under direction of nascent M12 RNA strands (*b*), (*d*), (*f*), (*h*) and phage M12 RNA (*k*). - - -, ^{3}H-labelled protein synthesized in the absence of exogenous RNA. ○—○ and □—□, ^{3}H-labelled proteins synthesized in presence of M12 coat protein (*c*), (*k*). △—△, ^{35}S-labelled proteins synthesized in actinomycin C_3 treated cells infected with the phage M12 and used as markers in (*d*) and (*i*). The radioactivity from control samples has been subtracted. Arrows at R, A and C refer to locations of complete replicase, *A*-protein and coat protein, respectively. The electrophoresis pattern of the proteins isolated from M12 phage, known to contain only *A*-protein and coat protein[18], can be superimposed on their respective peaks (unpublished results). Phage M12 RNA and nascent RNA strands, used to direct the cell-free protein synthesis (10–25 μg/assay tube, 60 μl. total incubation volume), were isolated from purified phage[23] and M12 replicative intermediate (Table 1), respectively. The M12 coat protein was isolated from purified phage with 66 per cent acetic acid. The centrifuged supernatant was dialysed overnight against 0·001 M acetic acid before being used in repression studies[14]. The cell-free system of *E. coli* strain AB301 (λ), met$^-$ and ribonuclease I$^-$, was prepared as described[19], but before being stored in liquid nitrogen the preincubated extract (30 min at 37° C) was dialysed for 5 h at 2° C against three changes of 0·05 M Tris-HCl (*p*H 7·8), 0·01 M magnesium acetate, 0·06 M NH_4Cl, 0·006 M 2-mercaptoethanol. The reaction mixture contained the components described by Capecchi[20]. Incubations (30 min at 37° C) were performed in the presence of ^{3}H-leucine (10 nmole/ml., 5 Ci/mmole) and ^{3}H-histidine (2 nmole/ml., 40 Ci/mmole). Where indicated, coat protein was added to the RNA before incubation (molar ratio of coat protein/RNA, approximately 40). The reaction was terminated by the addition of one-fourth volume of 0·25 M Tris-HCl buffer, *p*H 7·8, containing 5 per cent SDS, 0·25 M EDTA and 0·7 M 2-mercaptoethanol and vigorous shaking. An equal volume of phenol, saturated with this buffer, was added. The mixture was shaken for 5 min at room temperature and centrifuged for 10 min at 10,000 r.p.m. The phenol layer was removed and the extraction procedure was repeated. To precipitate the proteins, two volumes of acetone containing 0·1 M acetic acid were added to the combined phenol layers which were left to stand for 30 min in an ice bath. The precipitate was centrifuged (10 min at 10,000 r.p.m.), washed once with 5 ml. ether–ethanol (1 : 1) and once with 5 ml. ether, dried and then dissolved in a small volume of 0·01 M sodium phosphate (*p*H 7·2), containing 0·1 per cent SDS, 0·14 M 2-mercaptoethanol and 4 M urea. This solution was dialysed for 24 h against 500 volumes of the same buffer. The dialysing solution was changed twice. To 160 μl. of the dialysed solution, 40 μl. glycerol and 1 μl. 0·2 per cent bromophenol blue were added. This was layered onto 10 per cent polyacrylamide diacrylate gels (length 6·5 cm, diameter 0·6 cm), prepared in the presence of 4 M urea according to Viñuela *et al.*[3]. The electrophoresis buffer was 0·1 M phosphate (*p*H 7·2), containing 0·1 per cent SDS, 0·14 M 2-mercaptoethanol and 4 M urea. Electrophoresis was at room temperature for 12 h, 5 mA/gel. When the marker had reached the bottom of the tube the gels were removed and sliced, without freezing, into 1 mm sections. The slices were placed in scintillation vials and dissolved, with shaking, overnight at 37° C in 1 ml. of a solution consisting of 10 per cent piperidine and 10 per cent 'Hyamine 10X'. 15 ml. Kinard's scintillation solution[21] was added to each vial and the samples were counted in a Packard 'Tri-Carb' liquid scintillation counter. The procedure described by Oeschger and Nathans[5] for the preparation of phage specific proteins from infected cells was followed. Cells were grown in 'M9' medium[22] containing $MgCl_2$ in place of $MgSO_4$. $^{35}SO_4$ was added to label the proteins and actinomycin C_3 was used instead of actinomycin D (final concentration: 6 μg/ml.). 40 min after infection the cells were lysed by the method described by Fromageot and Zinder[23]. The proteins were isolated in essentially the same manner as the *in vitro* products.

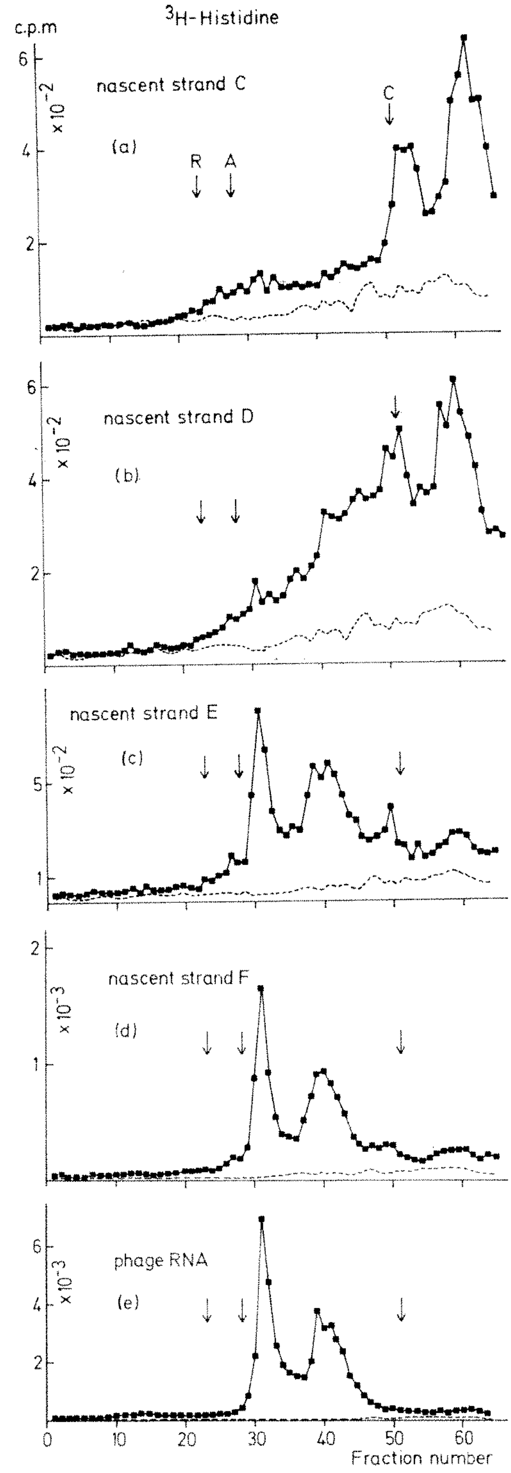

Fig. 2. Polyacrylamide gel electrophoresis of proteins made in the cell-free extract of *B. stearothermophilus* in the presence and absence of nascent M12 RNA strands. ■—■, ^{3}H-histidine labelled proteins synthesized under the direction of nascent M12 RNA strands (*a*), (*b*), (*c*), (*d*) and phage M12 RNA (*e*). - - -, ^{3}H-histidine labelled proteins synthesized in the absence of exogenous RNA. Arrows marked R, A and C refer to locations of complete replicase, *A*-protein and coat protein, respectively. *B. stearothermophilus* strain NU10 (a gift of Dr W. Zillig) was grown at 65° C in the medium described by Friedman and Weinstein[27]. The preparation of the cell-free system and the composition of the incubation mixture were as described in Fig. 1. All incubations were at 55° C for 20 min in the presence of ^{3}H-histidine (2 nmole/ml., 40 Ci/mmole). No extra tRNA was included. The method of protein isolation and the procedure of polyacrylamide gel electrophoresis are described in the legend to Fig. 1.

synthesized under the direction of the *A*-protein gene. Consequently, that gene must be located near the 5′ end and be followed by the coat protein gene and, finally, by the replicase gene. Although there are indications that complete *A*-protein is made in the *E. coli* system[34], no sharp *A*-protein peak can be demonstrated in the gel patterns, so that no direct evidence for the localization of the *A*-protein gene can be derived from these experiments.

In a cell-free system derived from *Bacillus stearothermophilus*, synthesis of only the phage *A*-protein is initiated[25,26]. If the gene order we postulate is correct, the small nascent strands (C and D) should initiate protein synthesis in this system. Fig. 2 shows that strand C stimulates some protein synthesis and must therefore contain the initiation site of the *A*-protein gene. Furthermore, strand D directs the synthesis of material with a higher molecular weight than that stimulated by strand C, and the *A*-protein gene is essentially complete in strand E, for its translational product is almost identical to that directed by complete phage RNA. When equal molar quantities of strands E and F are used, F directs the synthesis of about twice as much protein as E in this system. The difference in coat protein synthesis by equal molar quantities of these strands in the *E. coli* system (Figs. 1*c* and 1*d*) is, however, more than ten-fold. Thus strand E synthesizes relatively much more *A*-protein than coat protein, confirming that the *A*-protein gene precedes the coat protein gene.

It should be noted that even the largest peptide synthesized in the *B. stearothermophilus* system migrates slightly faster during gel electrophoresis than *A*-protein synthesized *in vivo*. This indicates that *A*-protein synthesis is somehow interrupted in this system. Comparable results were found by Lodish[26], who has shown that, although all tryptic peptides of *A*-protein labelled with methionine are made in this cell-free system, much less of the tryptic peptide located nearest to the C-terminus of the molecule is synthesized relative to the peptide fragments found nearer the N-terminus.

The protein products synthesized *in vitro* by the nascent strands (Figs. 1 and 2) can be directly correlated with their average informational contents (see Table 1), as shown in the map of Fig. 3. The finding that nascent strands A and B, containing approximately fifteen and seventy-five nucleotides respectively, do not stimulate protein synthesis agrees with the report that the first initiation codon is not located within the first sixty nucleotides nearest to the 5′ end[25,28] of the RNA molecule. The hypothesis that a fourth gene[29,30] exists in phage RNA is not supported by our results—there would be insufficient space for a gene of usual size. We cannot, however, exclude the possibility that the nucleotide sequences between the known genes or the base sequences at the 5′ and 3′ ends of the RNA molecule are involved in the synthesis of small peptides. No termination codon has been detected within the sequence of nucleotides at the 3′ end studied so far[28].

In view of biochemical similarities we conclude, finally, that the gene order we describe here also applies to the other members of the f2 group to which M12 is serologically related[31,32].

Implication of the Gene Sequence for Regulation

As can be seen in Fig. 1*i* and *k*, the phage *A*-protein gene is much less frequently translated than the coat protein gene. In the light of the gene order, this difference in efficiency of translation can no longer be explained by polarity mechanisms as previously suggested[6-8]. Apparently—at least for polycistronic phage RNA messenger—other regulatory mechanisms must be postulated, for example, initiation sites of different specificities[25].

Coat protein represses the translation of the phage replicase gene. In addition (Fig. 1*c*), the synthesis of complete *A*-protein can also be repressed, which agrees

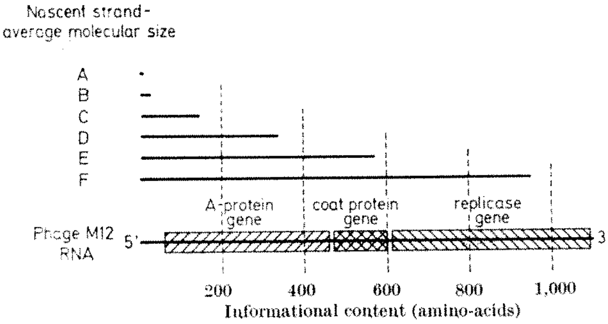

Fig. 3. Linear model of M12 RNA, showing the gene order, the approximate gene size, and the informational content of the complete M12 RNA molecule in relation to the incomplete M12 nascent strands. The informational contents of the nascent strands and of complete phage RNA are given in Table 1. Molecular weights—and amino-acid contents—of *A*-protein, coat protein and replicase were estimated from the results of polyacrylamide gel analyses (unpublished). The beginning of the *A*-protein gene is not made to coincide with the 5′ end of the phage RNA molecule, for it is known that nascent strands A and B do not stimulate protein synthesis and no initiation site occurs within the first sixty nucleotides from the 5′ end[25,28]. The reason for the gap shown between the coat protein and replicase genes is based on the finding that the termination codon for coat protein and the initiation codon for the replicase are not closely linked[24,25]. Similarly, a discontinuity is assumed to occur between the *A*-protein gene and the coat protein gene. As described in the text, the end of the replicase gene must be near to the 3′ end of the RNA molecule. Because no termination codon has so far been found in the nucleotides located near the 3′ end of this molecule[28], the end of the replicase gene is not placed directly at the terminus of the RNA genome.

with the findings of most other investigators[14-17], although not with those of Lodish and Robertson[34]. But because it has also been shown[6,7] that the initiation of *A*-protein synthesis cannot be repressed, we conclude, tentatively, that coat protein represses only a part of the *A*-protein gene. This would also explain why a small but constant histidine containing peptide is synthesized in the *E. coli* cell-free system under the direction of repressed M12 RNA (Fig. 1*k*). Because coat protein probably represses two phage genes which are separated by a third gene, these coat protein molecules must therefore act at two or more different sites, either by binding directly to specific sites or by modifying the secondary structure of the RNA molecule.

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Cytoplasmic Methionine Transfer RNAs from Eukaryotes

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Eukaryotic cells contain two species of methionine accepting tRNA. One of these (Met-tRNA$_{F*}$) only incorporates methionine into N-terminal positions of polypeptides whereas the other (Met-tRNA$_{M*}$) donates methionine into internal positions. It is suggested that tRNA$_{F*}^{Met}$ is an initiator tRNA on 80S ribosomes.

TRANSFER RNA from bacteria contains two methionine accepting species tRNA$_F^{Met}$ and tRNA$_M^{Met}$[1]. Methionine attached to tRNA$_F^{Met}$ can be enzymatically formylated to give formylmethionyl tRNA$_F$ (fMet-tRNA$_F$), while methionyl tRNA$_M$ (Met-tRNA$_M$) does not accept formyl groups[2]. fMet-tRNA$_F$ is the chain initiator on the 70S ribosomes found in all prokaryotes and prokaryotic-type organelles (that is, chloroplasts and mitochondria)[3]. A distinguishing feature of the initiator tRNA is that Met-tRNA$_F$, even when not formylated, donates methionine exclusively into N-terminal positions of newly synthesized proteins; whereas Met-tRNA$_M$, in common with all other amino-acyl-tRNAs, donates its amino-acid into internal positions[4]. The failure of Met-tRNA$_F$ to incorporate methionine into internal positions is probably a result of its inability to form a complex with transfer factor T and GTP[5]. This, together with other unusual properties such as unique codon–anticodon recognition[1,6], and the specific interaction with the initiation factors[7], implies that the structure of tRNA$_F^{Met}$ has been specially adapted for its role as the initiator tRNA.

The mechanism of initiation on 80S type ribosomes is unknown. There are no confirmed reports of fMet-tRNA in the cytoplasm of eukaryotes. Cytoplasmic tRNA from guinea-pig liver[8] and yeast[9], however, contains two major methionine accepting tRNAs. As we have indicated, such systems do not contain transformylase activity, but one of the two Met-tRNAs can be formylated by *E. coli* transformylase[8,9]. Further experiments have revealed that cytoplasmic fMet-tRNA from such systems functions as a chain initiator with the same requirements and properties as *E. coli* fMet-tRNA$_F$ in the *E. coli* cell-free system[3,8,9]. Thus many of the unique structural features of *E. coli* tRNA$_F^{Met}$ have been retained in one of the

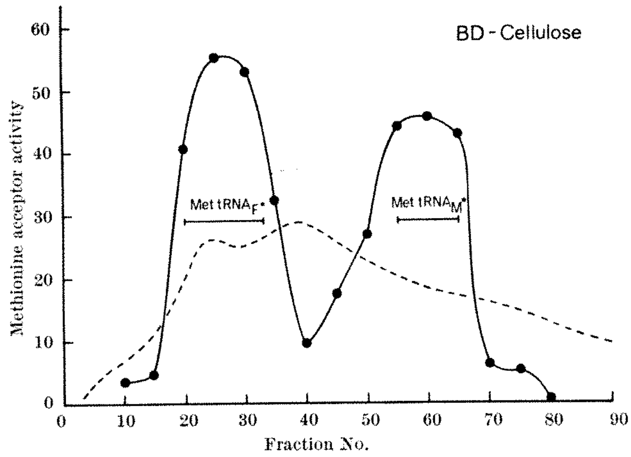

Fig. 1. Chromatography of partially purified tRNA$^{\mathrm{Met}}$ from ascites tumour cells on benzoylated DEAE cellulose. Approximately 100 A_{260} units of partially purified tRNA$^{\mathrm{Met}}$ from ascites tumour cells were applied to a 9 × 1 cm column of BD cellulose. The column was developed by a linear gradient formed from 200 ml. of 0·45 M NaCl, 0·01 M $MgCl_2$ and 0·001 M β-mercaptoethanol and 200 ml. of a similar solution containing 0·9 M NaCl. The flow rate was 10 ml./h and 2 ml. fractions were collected. ●—●, Methionine acceptor activity plotted in arbitrary units; - - - -, A_{260}.

Met-RNAs from yeast and guinea-pig liver, although the primary sequences of *E. coli* tRNA$^{\mathrm{Met}}_{\mathrm{F}}$ and the corresponding species in these systems are different[9,10].

We report here the separation of two methionine accepting tRNAs (tRNA$^{\mathrm{Met}}_{\mathrm{F^*}}$ and tRNA$^{\mathrm{Met}}_{\mathrm{M^*}}$) from mouse ascites tumour cells, mouse liver and yeast. tRNA$^{\mathrm{Met}}_{\mathrm{F^*}}$ is defined as that species which can be formylated by *E. coli* transformylase. We further demonstrate that Met-tRNA$_{\mathrm{F}}$* incorporates methionine exclusively into N-terminal positions of newly synthesized polypeptides in a cell-free system derived from mouse ascites tumour cells. Similar experiments with Met-tRNA$_{\mathrm{M}}$* revealed that methionine from this species is incorporated into internal positions only. This strongly suggests that mammalian transfer factor T-I discriminates against cytoplasmic Met-tRNA$_{\mathrm{F}}$*, just as bacterial transfer factor T discriminates against Met-tRNA$_{\mathrm{F}}$. Furthermore, the incorporation of methionine from Met-tRNA$_{\mathrm{F}}$* only into N-terminal positions indicates a special interaction between this tRNA and 80S ribosomes similar to that between 70S type ribosomes and bacterial tRNA$^{\mathrm{Met}}_{\mathrm{F}}$. These results are therefore taken to imply that Met-tRNA$_{\mathrm{F}}$* is a chain initiator on 80S type ribosomes, though not necessarily the only one.

Separation of Met-tRNAs

Krebs IIA mouse ascites tumour cells were propagated in male Schofields strain mice as previously described[11]. Pre-incubated S30 was made from washed cells by the method of M. B. Mathews and A. Korner (manuscript in preparation). Ribosomes were pelleted from the S30 by centrifugation at 150,000*g* for 90 min. The supernatant (S150) was then passed through a column of 'Sephadex *G*25' equilibrated with buffer *A* (120 mM KCl, 20 mM Tris HCl *p*H 7·5, 5 mM MgOAc, 6 mM β-mercaptoethanol) and stored in aliquots in liquid nitrogen. Ribosomes were re-pelleted through 1 M sucrose in buffer *A*, resuspended in buffer *A* and stored in liquid nitrogen. The preparation of encephalomyocarditis (EMC) virus and viral RNA has been described[11]. Amino-acyl-tRNA synthetases were prepared from the post ribosomal supernatants of mouse ascites cells, mouse liver and yeast by 40–70 per cent ammonium sulphate fractionation; when necessary endogenous tRNA was removed by passage through DEAE cellulose columns. Transfer RNA was isolated from Krebs IIA mouse ascites tumour cells and mouse liver, by repeated extraction of whole cells and liver with water-saturated phenol, followed by ethanol precipitation of the aqueous phase. Yeast tRNA was obtained commercially. The crude tRNAs were chromatographed on DEAE 'Sephadex'[9,12]. This gave little or no fractionation of the Met-tRNAs but led to an approximately four-fold increase in specific activity. The Met-tRNAs were separated by further chromatography on benzoylated DEAE (BD) cellulose. Fig. 1 shows the elution profile of ascites cell Met-tRNAs previously purified on DEAE 'Sephadex', on BD cellulose. Clearly two methionine accepting species are separated on this column. Identical elution profiles and separation of Met-tRNAs were obtained by chromatography of partially purified Met-tRNA from mouse liver and yeast.

Fig. 2 shows an autoradiograph of an ionogram of a ribonuclease T_1 digest of the two Met-tRNAs charged with ^{35}S-methionine by the homologous amino-acyl-tRNA synthetase in the presence and absence of *E. coli* transformylase. The two Met-tRNAs have different nucleotide sequences near the amino-acid acceptor end and only the methionyl-oligonucleotide from Met-tRNA$_{\mathrm{F}}$* is converted to the formyl-derivative when charged in the presence of *E. coli* transformylase. Similar digestion patterns were obtained for the separated Met-tRNAs from mouse liver and yeast. In all cases the degree of cross-contamination was less than 5 per cent. The terminal sequence of yeast Met-tRNA$_{\mathrm{F}}$* is CUACCA-Met[9]. Because the corresponding

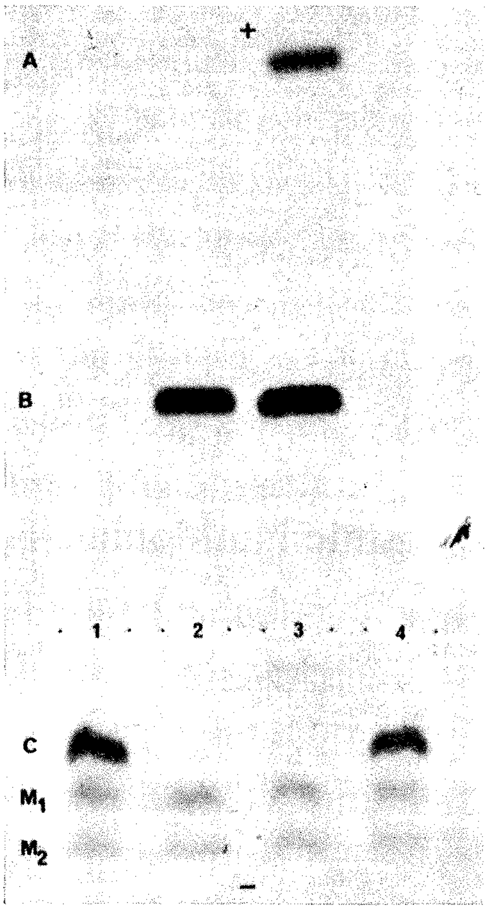

Fig. 2. Autoradiograph of an ionogram of T_1 ribonuclease digests of separated Met-tRNAs from ascites cells. Separated Met-tRNAs from ascites tumour cells were charged with ^{35}S-methionine by crude homologous aminoacyl-tRNA synthetases in the presence and absence of crude *E. coli* supernatant and formyl donor. After re-isolation the charged tRNAs were digested with T_1 ribonuclease (1 : 50)[2]. After 20 min at 37° C the samples were applied to Whatman 3 *MM* paper and electrophoresed at *p*H 3·5 for 3 h. 1, Digest of ^{35}S-Met-tRNA$_{\mathrm{M}}$* charged by ascites enzyme only; 2, digest of ^{35}S-Met-tRNA$_{\mathrm{F}}$* charged as for 1; 3, digest of ^{35}S-Met-tRNA$_{\mathrm{F}}$* charged by ascites enzyme in the presence of *E. coli* supernatant and formyl donor; 4, digest of ^{35}S-Met-tRNA$_{\mathrm{M}}$* charged as for 3. *A*, A formyl methionine containing oligonucleotide; *B* and *C*, methionine containing oligonucleotides. M_1 and M_2, methionine sulphoxide and methionine respectively.

methionyl-oligonucleotides from ascites cell and mouse liver Met-tRNA$_F$*s have mobilities identical to the yeast oligonucleotide it is probable that the terminal sequence of these tRNAs is also CUACCA$_{OH}$. It is interesting that the formyl methionyl-oligonucleotide obtained from rat liver mitochondrial fMet-tRNA by ribonuclease T$_1$ digestion also has the same mobility as that obtained from enzymatically formylated Met-tRNA$_F$* from the corresponding cytoplasm.

Although separation was not attempted we have further found that rat liver and rabbit reticulocyte tRNA contain a methionine tRNA that can be formylated by *E. coli* transformylase. A similar result has also been reported for tRNA isolated from the protozoan, crithidia (G. Cross, personal communication). These findings therefore indicate that many, perhaps all, eukaryotic cells contain a cytoplasmic species of Met-tRNA that is recognized by *E. coli* transformylase.

Methionine Incorporation in Cell-free Systems

For incorporation studies using the separated Met-tRNA species we used a cell-free system prepared from mouse ascites tumour cells. All experiments were carried out at 5 mM Mg^{2+} concentration, which is the optimum for endogenous and EMC RNA translation[13]. Table 1 records the results obtained using synthetic polymers and EMC RNA. Met-tRNA$_M$* donates methionine into polypeptides in response to random poly-AUG better than does Met-tRNA$_F$* whereas the reverse is the case for poly-UG. This is identical to the results obtained previously with similar polymers in the *E. coli* cell-free system using *E. coli* Met-tRNA$_F$ and Met-tRNA$_M$[1]. In the latter case Met-RNA$_F$ incorporated methionine into N-terminal positions only while Met-tRNA$_M$ incorporated methionine into internal positions. Accordingly, end group determinations on the polypeptides synthesized in the mammalian cell-free system were carried out by the Edman procedure[14]. From the results shown in Table 2 it is evident that methionine from Met-tRNA$_F$* is N-terminal while that from Met-tRNA$_M$* is internal. This result was further confirmed using the blocked polymers AUG(U)$_n$ and GUG(U)$_n$ (where n is about 30). As Table 1 shows, only Met-tRNA$_F$* donates methionine in response to these polymers, Met-tRNA$_M$* being totally inactive in these conditions.

Table 1. INCORPORATION OF ^{35}S-METHIONINE FROM SEPARATED Met-tRNAs FROM ASCITES TUMOUR CELLS

	*p*Moles of ^{35}S methionine incorporated from (1) ^{35}S-Met-tRNA$_F$*	(2) ^{35}S-Met-tRNA$_M$*
(*A*) Additions		
None	0·02	0·11
30 μg of poly-AUG (1 : 2·5 : 1)	0·26	0·60
25 μg of poly-UG (1 : 1)	0·84	0·31
17 μg of AUG(U)n	0·61	0·18
15 μg of GUG(U)n	0·67	0·10
4 μg of EMC RNA	0·12	1·19
(*B*) Endogenous RNA	0·28	1·58

(*A*) Incorporations were carried out in a total of 50 μl. containing 20 mM Tris HCl *p*H 7·5, 120 mM KCl, 5 mM Mg^{2+}, 6 mM β-mercaptoethanol, 0·09 mg of creatine phosphate, 10 μg of creatine phosphokinase, 1 mM ATP, 0·1 mM GTP and 25 μM of each of the twenty amino-acids. All incubations contained 0·2 mg of S150 protein and 50 μg of ribosomes. 3·5 jsMoles of either of the separated ascites cell ^{35}S-Met-tRNAs were added and incubation continued for 30 min at 37° C. Reactions were stopped by addition of 5 per cent TCA containing 4 per cent casamino-acids and subsequently heated for 15 min at 90° C. The samples were collected on glass fibre paper, washed, and counted in a scintillation counter at 60 per cent efficiency. 1 *p*Mole equals 26,400 c.p.m. (*B*) Incorporations using a non-preincubated ascites cell S30 preparation which contained the same ingredients as for (*A*) except that 0·25 mg of non-preincubated S30 was added instead of S150 and ribosomes.

Table 2. END GROUP DETERMINATION OF ^{35}S METHIONINE LABELLED POLYPEPTIDE PRODUCT SYNTHESIZED IN RESPONSE TO RANDOM POLY AUG AND POLY UG

Polymer	Per cent N-terminal incorporation from (1) ^{35}S-Met-tRNA$_F$*	(2) ^{35}S-Met-tRNA$_M$*
poly-AUG	89	3
poly-UG	95	5

Incorporations were carried out in a total of 100 μl., in the conditions described in Table 1. After 30 min at 37° C, 100 μl. of 100 mM EDTA containing 10 μg of pancreatic ribonuclease was added and incubation continued for 2 min. The samples were precipitated with 5 per cent TCA containing 4 per cent casamino-acids, washed several times with 5 per cent TCA, and finally dried. End group determinations were then carried out using a modified Edman procedure[14]. After exposure to trifluoroacetic acid the thiazolinones were extracted with 1,2-dichloroethane. The resulting organic phase was evaporated to dryness, taken up in formic acid and aliquots taken for counting. The residual protein was partially hydrolysed in 6 N HCl, dried and finally dissolved in formic acid; aliquots were then taken for counting.

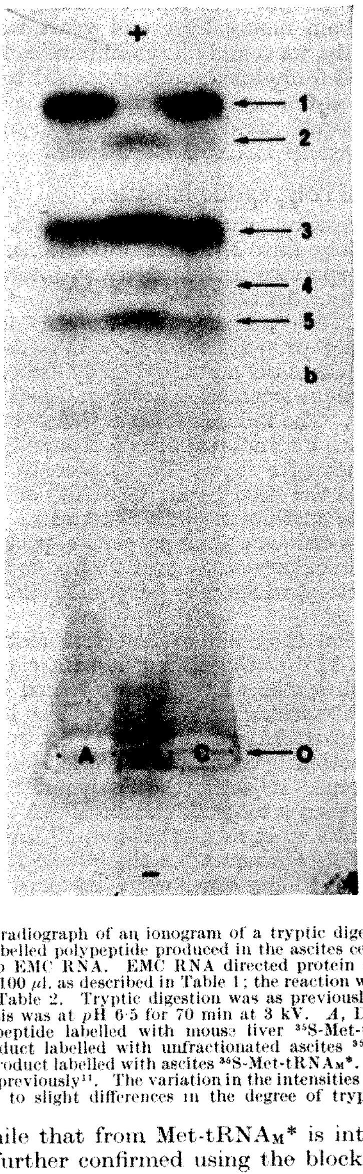

Fig. 3. Autoradiograph of an ionogram of a tryptic digest of the ^{35}S-methionine labelled polypeptide produced in the ascites cell-free system in response to EMC RNA. EMC RNA directed protein synthesis was performed in 100 μl. as described in Table 1; the reaction was stopped as described in Table 2. Tryptic digestion was as previously described[11]. Electrophoresis was at *p*H 6·5 for 70 min at 3 kV. *A*, Digest of virus specific polypeptide labelled with mouse liver ^{35}S-Met-tRNA$_M$*; *B*, digest of product labelled with unfractionated ascites ^{35}S-Met-tRNA; *C*, digest of product labelled with ascites ^{35}S-Met-tRNA$_M$*. Peptides are numbered as previously[11]. The variation in the intensities of some of the bands is due to slight differences in the degree of tryptic digestion.

We have demonstrated that virus specific polypeptide(s) are synthesized in the ascites cell-free system primed by EMC RNA[11]. Subsequent investigation of the virus specific polypeptide product synthesized in these conditions has revealed that alanine is the N-terminal amino-acid. Thus all methionine residues in this product must be internal. Table 1 indicates that only Met-tRNA$_M$* incorporates methionine into virus specific polypeptide in response to EMC RNA. Very little, if any, incorporation is detected using Met-tRNA$_F$*. This conclusion was further confirmed by comparing a tryptic digest of the virus specific product labelled by unfractionated Met-tRNA with a similar digest of the product labelled by Met-tRNA$_M$* only. Fig. 3 shows that Met-tRNA$_M$* donates all the methionine residues normally found in the product made using unfractionated tRNA. If methionine from Met-tRNA$_F$* is incorporated into the N-terminal position of the virus specific protein, then it must be rapidly removed again, presumably by an aminopeptidase.

Endogenous protein synthesis in non-preincubated S30 preparations from ascites tumour cells with the separated Met-tRNAs gave essentially similar results (Table 1).

All these experiments were repeated using the separated Met-tRNAs from mouse liver and yeast with identical results. Finally, we would like to emphasize that we have never detected formylmethionine attached to tRNA$^{Met}_{F*}$ in the ascites cell-free system, nor has it been found in polypeptides synthesized in response to synthetic polymers[15], endogenous RNA or EMC RNA.

Mechanism of Polypeptide Initiation

Our experiments show that Met-tRNA$_F$* donates methionine only into N-terminal positions, while Met-tRNA$_M$* incorporates methionine into internal positions of polypeptides. In addition, Met-tRNA$_M$* responds to the codon AUG[8,9,16], whereas Met-tRNA$_F$* has the same unusual coding properties as *E. coli* fMet-tRNA$_F$, responding both to AUG and GUG on 80S ribosomes. This indicates a unique interaction between this tRNA and the 80S ribosome. The failure of Met-tRNA$_F$* to incorporate methionine into internal positions presumably means that mammalian transfer factor T-I is able to discriminate against it. On the other hand, the ability of Met-tRNA$_F$* to incorporate methionine into N-terminal positions, in addition to its unique coding properties, probably reflects the presence in eukaryotic cells of a separate set of factors (initiation factors) which may be specific for Met-tRNA$_F$* only.

In conclusion the unique structural features of prokaryotic tRNA$^{Met}_F$ distinguishing it as a chain initiating tRNA in such systems have been retained in the corresponding species in higher organisms. The interaction of eukaryotic Met-tRNA$_F$* and the 80S ribosome is identical to the interaction between prokaryotic Met-tRNA$_F$ and the 70S ribosome. Rejecting the highly unlikely possibility that tRNA$^{Met}_{F*}$ is a totally redundant tRNA in eukaryotic cells, our results must mean that Met-tRNA$_F$* is a chain initiator on 80S ribosomes for the simple reason that it cannot function in any other way. At the same time the notion that any amino-acyl-tRNA can initiate on 80S ribosomes can also be rejected because Met-tRNA$_M$* clearly cannot do so. The possibility that Met-tRNA$_F$* is merely a source for mitochondrial fMet-tRNA$_F$ also seems unlikely because all the experiments described here have been performed on cytoplasmic ribosomes. In addition, the amount of cytoplasmic tRNA$^{Met}_{F*}$ (that is, 1–2 per cent of total tRNA) is vastly greater than that found in the mitochondria.

Thus there seem to be three likely models for initiation on 80S ribosomes. First, Met-tRNA$_F$* without the formyl group is the only chain initiator on 80S ribosomes, and incorporates methionine into the N-terminal position of all nascent proteins. If this is so, the methionine must be removed again in most cases, since it is not a common N-terminal amino-acid in eukaryotes. Further, we have not so far detected any N-terminal methionine in the product synthesized in response to EMC RNA, nor has it been reported in rabbit reticulocyte haemoglobin synthesis. Such a theory would make initiation on 80S ribosomes virtually identical to that on 70S ribosomes, the only difference being the lack of formylation and a slight modification in the specificity of cytoplasmic initiation factors.

Second, Met-tRNA$_F$* could select the reading frame only and not be incorporated into proteins. Met-tRNA$_F$* can incorporate methionine in response to synthetic polymers but the situation might be different with natural messenger RNAs. Earlier experiments with rabbit reticulocyte cell-free systems have shown that modified valyl-tRNA is incorporated into the N-terminal position of nascent haemoglobin[17]. These two models for initiation can explain these results by arguing that in such systems the messenger RNA is always attached to the ribosomes and thus the first step in initiation involving Met-tRNA$_F$* may already be completed.

Third, a class of cytoplasmic tRNAs might exist with structural features identical to tRNA$^{Met}_{F*}$. Such tRNAs would thus be specifically adapted for their role as chain initiators.

We thank Mr M. J. Young for technical assistance, and Dr J. Brown for blocked oligonucleotide polymers. A. E. S. is an MRC scholar.

After completion of our manuscript we noted a recent paper by Takeishi, Sekiya and Ukita (*Biochim. Biophys. Acta*, **199**, 559; 1970), who have shown that only yeast and *E. coli* Met-tRNA$_M$ donate methionine into haemoglobin synthesized in a rabbit reticulocyte cell-free system.

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Initiator Codons in Eukaryotes

by

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A unique interaction between eukaryotic Met-tRNA$_{F*}$, AUG and 80S ribosomes is demonstrated. It is suggested that tRNA$^{Met}_{F*}$ is the only initiator tRNA on 80S ribosomes and that AUG and possibly GUG are the initiator codons.

THE cytoplasm of several eukaryotes contains two methionine accepting tRNAs (tRNA$^{Met}_{F*}$ and tRNA$^{Met}_{M*}$)[2]. Methionine attached to tRNA$^{Met}_{F*}$ can be formylated by *Escherichia coli* transformylase[1], while Met-tRNA$_{M*}$ does not accept formyl groups. In an *E. coli* cell-free system eukaryotic fMet-tRNA$_{F*}$ functions as an initiator with the same requirements and specificity as *E. coli* fMet-tRNA$_F$ (refs. 2 and 3). The preceding article demonstrates that in a mammalian cell-free system Met-tRNA$_{F*}$ donates methionine exclusively into N-terminal position$_*$ of newly synthesized polypeptides, while Met-tRNA$_{MS}$ donates methionine only into internal positions[2]. Thus discrimination against the Met-tRNA$_{F*}$ type of species by the corresponding aminoacyl-tRNA binding enzymes

(T on 70S and T–I on 80S) seems to be identical for the two ribosomal systems[2,4]. This suggests that Met-tRNA$_{\mathrm{F}*}$ is a chain initiator on 80S ribosomes, although not necessarily the only one. Here we show that the interaction between Met-tRNA$_{\mathrm{F}*}$, AUG and the 80S ribosome is unique, closely resembling the corresponding interaction between prokaryotic fMet-tRNA$_{\mathrm{F}}$, AUG and the 70S ribosome. (The term 80S ribosome is adopted for convenience: it is quite probable that the interaction described here first occurs on the 40S ribosomal subunit.) No other aminoacyl-tRNA tested shows a similar interaction with its corresponding codon on the 80S ribosome. These results therefore imply that Met-tRNA$_{\mathrm{F}*}$ is the only chain initiator tRNA on 80S ribosomes and that AUG and possibly GUG are the only initiator codons in eukaryotes.

Propagation of Krebs II mouse ascites tumour cells, preparation of the cell-free system, and fractionation of the tRNA were performed as previously described[2]. Synthetic polynucleotide mRNAs were prepared by established procedures using the enzyme polynucleotide phosphorylase[5]. The base sequence of the products was verified by ribonuclease digestion followed by identification of the digestion products by chromatography.

AUG as Initiator Codon

In a cell-free system derived from ascites tumour cells, Met-tRNA$_{\mathrm{F}*}$ donates the methionine incorporated into the polypeptide product synthesized in response to AUG(U)$_{\bar{n}}$($\bar{n}$ = 35)[2]. No incorporation of methionine from Met-tRNA$_{\mathrm{M}*}$ is detected. Similarly, only Met-tRNA$_{\mathrm{F}*}$ donates methionine in response to GUG(U)$_{\bar{n}}$[2]. Further experiments have now shown that there is virtually no incorporation of valine in these conditions, although there is a very efficient incorporation of valine in response to random poly(U,G). Thus, when presented with an AUG or GUG at the 5′ end of a synthetic messenger RNA, the 80S ribosome interacts only with Met-tRNA$_{\mathrm{F}*}$ and discriminates against Met-tRNA$_{\mathrm{M}*}$ and Val-tRNA.

Table 1 records the results obtained using a series of polymers in which the AUG was located at different distances from the 5′ end. In all cases methionine is donated by Met-tRNA$_{\mathrm{F}*}$. That very little incorporation from Met-tRNA$_{\mathrm{M}*}$ or Lys-tRNA could be detected further indicates that synthesis is initiated almost exclusively at the AUG position. Thus an AUG at or near the 5′ end of synthetic mRNA signifies Met-tRNA$_{\mathrm{F}*}$. Furthermore, the interaction between Met-tRNA$_{\mathrm{F}}$* and this particular AUG results in a phasing effect; the mRNA is read almost exclusively from AUG and not in other possible reading frames. The ability of the 80S ribosome to distinguish Met-tRNA$_{\mathrm{F}*}$ and not Met-tRNA$_{\mathrm{M}*}$ in response to an AUG in such a position most probably results from the presence of initiation factors which mediate this specific interaction. Similar arguments can be made for recognition of GUG as Met-tRNA$_{\mathrm{F}*}$ and not Val-tRNA.

In the presence of low Mg^{2+} concentrations only polymers containing AUG and GUG are translated in the *E. coli* cell-free system[6]. In such conditions, initiation on the 70S ribosome "by mistake" is apparently no longer possible. Synthesis in response to AUG and GUG polymers then strictly depends on the presence of initiation factors and fMet-tRNA$_{\mathrm{F}}$. Removal of the initiation factors completely abolishes synthesis[6]. Omission of the formyl group on Met-tRNA$_{\mathrm{F}}$ also abolishes synthesis[7], because the initiation factors are specific for fMet-tRNA (ref. 8). They do not recognize Met-tRNA$_{\mathrm{F}}$, and consequently no interaction can then occur between this tRNA and the 70S ribosome. In such conditions polymers containing AUG behave identically to all other polymers.

Table 1. INCORPORATION OF ^{35}S-METHIONINE FROM SEPARATED Met-tRNAs FROM ASCITES TUMOUR CELLS

Additions	pmoles ^{35}S-methionine incorporated from (1) ^{35}S-Met-tRNA$_{\mathrm{F}*}$	(2) ^{35}S-Met-tRNA$_{\mathrm{M}*}$
None	0·05	0·16
AUG(U)$_{\bar{n}}$	0·52	0·11
AAUG(U)$_{\bar{n}}$	0·42	0·12
AAAUG(U)$_{\bar{n}}$	0·27	0·11
AAAAUG(U)$_{\bar{n}}$	0·30	0·14
AAAAAUG(U)$_{\bar{n}}$	0·28	0·13
A$_{10}$UG(U)$_{\bar{n}}$	0·27	0·19

Incorporations were carried out in a total of 50 µl. in the conditions described previously[2]. The Mg^{2+} concentration was 5 mM. 15·5 µg of oligonucleotide polymer was added as indicated, and 3·5 pmoles of either of the separated ^{35}S-Met-tRNAs added to each incubation. 1 pmole corresponded to 26,400 c.p.m. Small but measurable amounts of lysine were incorporated from ^{14}C-lysyl-tRNA with A$_5$UG(U)$_{\bar{n}}$ and A$_{10}$UG(U)$_{\bar{n}}$. $\bar{n}$ is in all cases approximately 35.

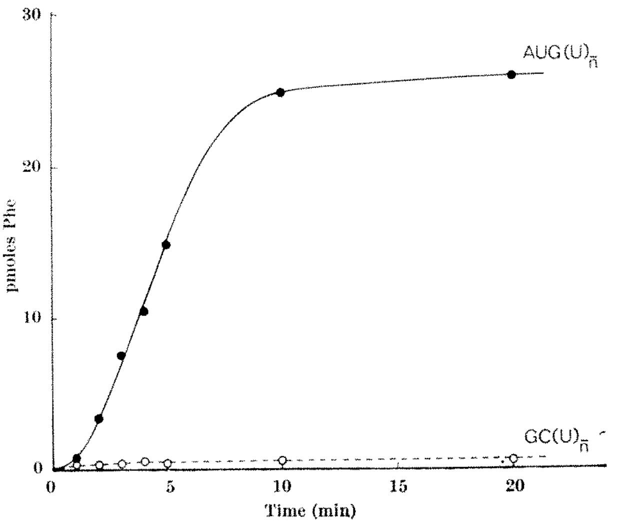

Fig. 1. Incorporation of phenylalanine in response to AUG(U)$_{\bar{n}}$ and GCU(U)$_{\bar{n}}$ in the ascites cell-free system at 2 mM Mg^{2+} concentration. Incorporations were carried out in a total of 250 µl. in conditions similar to those described previously[2]. The contents of each incubation included half the normal amount of ribosomes and S150, 0·6 µCi of ^{14}C-phenylalanine, 10 nmoles of each of the nineteen other amino-acids and 50 µg of synthetic polynucleotide. The Mg^{2+} concentration was 2·25 mM. 25 µl. aliquots were removed at the times indicated, and prepared for counting as previously described. 1 pmole corresponds to 660 c.p.m. The product synthesized in the presence of ^{35}S-methionine or ^{3}H-phenylalanine in response to AUG(U)$_{\bar{n}}$ was identified by digestion of the polypeptide with chymotrypsin, followed by electrophoresis of the digestion product at pH 3·5. The only ^{35}S-methionine labelled peptide co-electrophoresed with marker Met-Phe; the ^{3}H-phenylalanine-labelled peptides co-electrophoresed with Phe-Phe and Met-Phe ($\bar{n}$ is in both cases ≃35).

In the ascites cell-free system, conditions can be found where only synthetic polymers containing AUG are translated. Fig. 1 shows that at approximately 2 mM Mg^{2+} concentration AUG(U)$_{\bar{n}}$ is translated while GCU(U)$_{\bar{n}}$ is not. The incorporation in response to AUG(U)$_{\bar{n}}$ is at least 50 per cent of that obtained at the optimal Mg^{2+} concentration. In addition, AUG(U)$_{\bar{n}}$ has a lower Mg^{2+} optimum than that observed for all other polynucleotide mRNAs tested. Experiments using the polymers shown in Table 2 indicate that they all behave identically to GCU(U)$_{\bar{n}}$, and give no phenylalanine incorporation at 2 mM Mg^{2+} concentration. All polymers are active at 5 mM Mg^{2+} concentration, with activities being comparable with that of AUG(U)$_{\bar{n}}$. In these conditions, however, only AUG(U)$_{\bar{n}}$ and GUG(U)$_{\bar{n}}$ are initiated with high

Table 2. PHENYLALANINE INCORPORATION WITH DIFFERENT POLYNUCLEOTIDES

UUU(U)$_{\overline{35}}$
UUG(U)$_{\overline{35}}$
CUU(U)$_{\overline{35}}$
CUG(U)$_{\overline{35}}$
AUU(U)$_{\overline{35}}$
GUU(U)$_{\overline{35}}$
GUA(U)$_{\overline{35}}$
GUG(U)$_{\overline{35}}$
UCU(U)$_{\overline{35}}$
CCU(U)$_{\overline{35}}$
ACU(U)$_{\overline{35}}$
GCU(U)$_{\overline{35}}$
UAU(U)$_{\overline{35}}$
AAU(U)$_{\overline{35}}$
AAA(U)$_{\overline{35}}$
AAG(U)$_{\overline{35}}$
GAU(U)$_{\overline{35}}$
GAA(U)$_{\overline{35}}$
UGU(U)$_{\overline{35}}$
CGU(U)$_{\overline{35}}$
AGU(U)$_{\overline{35}}$

The above synthetic polynucleotides were tested in the ascites cell-free system in the conditions described in Fig. 1, and gave no incorporation of phenylalanine at 2 mM Mg^{2+}.

Table 3. INCORPORATION OF ^{35}S-METHIONINE FROM ASCITES fMet-tRNA$_{F*}$ AND Met-tRNA$_{F*}$ IN RESPONSE TO RANDOM POLY AUG IN THE *E. coli* AND ASCITES CELL-FREE SYSTEMS

Cell-free system	pmoles of ^{35}S methionine incorporated from (1) ^{35}S fMet-tRNA$_{F*}$	(2) ^{35}S Met-tRNA$_{F*}$
E. coli	0·53	0·71
Ascites	0·09	0·66

Incorporations were carried out in a total of 50 µl. in standard conditions. The Mg^{2+} concentration in the *E. coli* S30 was 11 mM and in the ascites system was 5 mM. 50 µg of random poly AUG was added to each incubation. Purified ascites tRNA$^{Met}_{F*}$ was charged in the presence of *E. coli* transformylase and formyl donor and subsequently pyrophosphorylysed to remove unformylated Met-tRNA. The degree of formylation was checked by pancreatic digestion followed by electrophoresis at *p*H 3·5. 2·6 pmoles of fMet-tRNA$_{F*}$ or 3·3 pmoles of Met-tRNA$_{F*}$ were added as indicated. 13,000 c.p.m. correspond to 1 pmole.

efficiency from the 5′ end. The remaining polymers incorporate only small amounts of the amino-acid corresponding to the codon at the 5′ end. This is comparable with the result obtained in the *E. coli* cell-free system using similar polymers[9]. Experiments with polymers which have a radioactive triplet at the 5′ end showed that they were not rapidly broken down in the cell-free system. Further, no difference in half life between ^{14}C-labelled AUG(U)$_{\bar{n}}$ and that of other polymers could be detected. Thus our results cannot be explained by preferential stability of AUG(U)$_{\bar{n}}$ towards ribonuclease digestion.

Formation of the Initiation Complex

At low Mg^{2+} concentrations, therefore, only polymers containing AUG are translated in this mammalian cell-free system. By analogy with the similar situation in *E. coli*, we submit that this must mean that initiation factors are present in our cell-free system and that these factors are specific for Met-tRNA$_{F*}$. fMet-tRNA$_{F*}$ formylated by *E. coli* transformylase is not active in the cell-free system as shown in Table 3, indicating that the 80S ribosome does not interact with blocked aminoacyl-tRNAs. It should be noted also that fMet-tRNA or any similar derivatives have never been detected in the cytoplasm of eukaryotic organisms[3].

Our results indicate that a special interaction occurs between Met-tRNA$_{F*}$, AUG and the 80S ribosome. This interaction is identical in all respects to that occurring in the formation of the initiation complex on the 70S ribosome with fMet-tRNA$_F$ and AUG (ref. 10). Thus an AUG codon at or near the 5′ end of an mRNA signifies Met-tRNA$_{F*}$ and not Met-tRNA$_{M*}$. Similarly, GUG at the 5′ end of the mRNA indicates Met-tRNA$_{F*}$ and not Val-tRNA. The 80S ribosome is therefore able to discriminate between these possibilities without confusion. In addition, the presence of an AUG sequence at or near the 5′ end of an mRNA selects the reading frame, translation of other possible codons being severely suppressed. At low Mg^{2+} concentrations only polymers containing an AUG codon are translated.

If 80S ribosomal systems contain initiation factors which are specific for Met-tRNA$_{F*}$, Met-tRNA$_{F*}$—without a formyl group—must be the only chain initiator in such systems, with AUG and possibly GUG as the initiator codons. A consequence of this model for eukaryotic initiation is that all nascent proteins contain a methionine residue in front of the normal N-terminal amino-acid. The methionine residue must, in most cases, be subsequently removed by an aminopeptidase, for methionine is not a common N-terminal amino-acid in eukaryotes. There is already some evidence for this hypothesis. In a recent study of initiator mutants in yeast[11], several isocytochrome *c* mutants were isolated which had methionine as an additional amino-acid at the N-terminal position. It was concluded that the isocytochrome *c* mRNA contains an AUG codon which precedes the codon for the normal N-terminal amino-acid of the mature protein. Furthermore, methionine is incorporated into the N-terminal position of cytochrome *c*, to be removed again later, thus exposing the normal N-terminal amino-acid, threonine. These studies are in agreement with the results presented here and in the previous article. The similarity of eukaryotic polypeptide chain initiation with the corresponding process on 70S ribosomes is thus almost complete, the only difference between the two systems being the lack of formylation of Met-tRNA$_{F*}$ and a slight alteration in the specificity of the initiation factors in the 80S ribosomal system.

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Double-stranded RNA synthesized in Animal Cells in the Presence of Actinomycin D

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Animal RNA synthesized in the presence of actinomycin D is 40 per cent resistant to ribonucleases A and T_1, unmethylated and polydisperse on sucrose density gradients. The ribonuclease resistant core possesses properties of double-stranded RNA although the base composition suggests that it is not a simple base-paired structure.

ACTINOMYCIN D selectively inhibits DNA dependent RNA synthesis by complexing with the DNA template. At high concentrations (1·0–5·0 µg/ml.) it has been shown to inhibit more than 95 per cent of RNA synthesis in animal cells[1,2]. RNA synthesized in the presence of actinomycin has been observed previously[3–7], but it has never been adequately characterized. We have studied the residual RNA synthesis occurring in the presence of actinomycin in a number of animal cell lines and in mammalian tissue. Our observations suggest that this material, though it

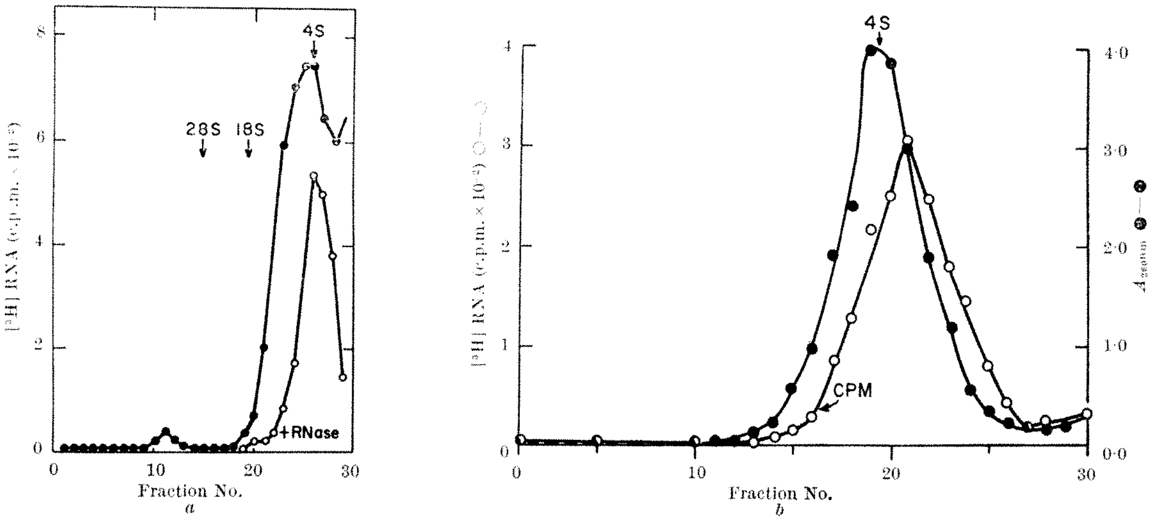

Fig. 1. Sucrose density gradient centrifugation of RNA synthesized in the presence of actinomycin in Burkitt lymphoma cells. RNA was dissolved in 2 × SSC (*a*) and divided into two aliquots. One was incubated with ribonuclease A (24 μg/ml. for 30 min at 37° C) followed by incubation with pronase (1·5 mg/ml. for 1 h at 37° C). The second aliquot was not treated. The two preparations were layered on separate tubes containing a 6 to 30 per cent gradient of sucrose, together with 0·1 ml. of rat liver cytoplasmic RNA containing four absorbance units at 260 nm, and sedimented for 50 min at 300,000*g*. Fractions were collected and analysed for absorbance at 260 nm and trichloroacetic acid precipitable ³H-RNA was determined as described in the text. The results from the two preparations are illustrated in the single figure. Arrows indicate the peak of absorbance of the rat liver RNAs. In *b* RNA treated with ribonuclease A and pronase was layered on a tube containing a 6 to 30 per cent gradient of sucrose together with marker *E. coli* B tRNA and sedimented at 300,000*g* for 5 h. Profiles of both absorbance of the tRNA marker and the trichloroacetic acid precipitable ³H-RNA are given.

has many of the attributes of a double-stranded RNA, is not a simple base-pair structure. RNA synthesis in the presence of actinomycin D was examined in animal cells from a wide variety of sources. ³H-uridine was incorporated into RNA in actinomycin treated cells at a rate which was 0·8–2·3 per cent of that found in untreated cells. Such incorporation was seen in all animal cells examined (Table 1). The size distribution of the RNA molecules synthesized by Burkitt lymphoma cells in the presence of actinomycin was examined by zone centrifugation on a sucrose density gradient (Fig. 1*a*). Labelled RNA was distributed between 4S and 14S with a peak at approximately 7S. Similar profiles were obtained for RNA from all the cell lines listed in Table 1.

RNA viruses replicate normally in actinomycin treated cells. This suggested that the RNA synthesized in the presence of actinomycin in our cells might have properties similar to those reported for viral RNA. The replicative cycle of RNA viruses is associated with ribonuclease-resistant structures[19]. We examined the susceptibility of the RNA synthesized in the presence of actinomycin to digestion by ribonucleases. As shown in Table 1, 40–68 per cent of the RNA remained acid insoluble after treatment with ribonuclease A. Incubation of this ribonuclease resistant core RNA with T_1 ribonuclease, deoxyribonuclease or pronase had no further effect on the level of acid precipitable RNA (Tables 2 and 3).

Table 1. LEVELS OF RNA SYNTHESIZED IN ANIMAL CELLS IN THE PRESENCE OF ACTINOMYCIN D

	A	B
Cell type	RNA (per cent of control)	RNA (per cent of A remaining after ribonuclease)
Cultured lymphoid cells		
Human peripheral lymphocytes in primary cultures		
Resting	1·2	48
24 h PHA stimulation	0·9	53
48 h PHA stimulation	1·9	63
Burkitt lymphona cells in continuous culture		
AL_1	2·8	57
AL_2	2·1	49
AL_3	2·7	56
Human lymphocytes in continuous culture		
Normal	1·9	55
Infectious mononucleosis	2·1	40
Hodgkin's disease	2·0	52
Chediak-Higashi syndrome	2·1	39
Cell lines in continuous culture, non-lymphoid		
Chick embryo fibroblasts	1·2	46
Chick embryo fibroblasts (sterile flock, RAV, RIF, RSV free)	2·7	60
AH-1	2·0	65
Osteogenic sarcoma	2·8	47
HeLa	2·8	58
Rat tissues		
Liver	0·8	38
Kidney	0·9	42
Spleen	0·8	47

Table 2. TREATMENT OF BURKITT LYMPHOMA CELL ³H-RNA SYNTHESIZED IN THE PRESENCE OF ACTINOMYCIN D

Treatment	Radioactivity (c.p.m.)
1. None	4,650
2. Ribonuclease A (24 μg/ml. for 1 h at 37° C in 2 × SSC)	1,950
3. As in 2 except in 0·1 × SSC	50
4. KOH (0·3 M for 16 h at 37° C)	30
5. As in 2 followed by pronase (1·5 mg/ml. for 1 h at 37° C)	1,800
6. Deoxyribonuclease (20 μg/ml. for 30 min at 37° C in 0·1 × SSC) followed by 2	1,900
7. As in 2 followed by heat (3 min at 100° C in 0·1 × SSC), rapid cooling and again treated with ribonuclease as in 2, in 2 × SSC	740
8. As in 2 followed by incubation with DMSO (90 per cent for 30 min at 37° C), dialysis; and again treated with ribonuclease as in 2, in 2 × SSC	725

Table 3. TREATMENT OF CHICK EMBRYO FIBROBLAST ³²P-RNA SYNTHESIZED IN THE PRESENCE AND ABSENCE OF ACTINOMYCIN D

Treatment	With actinomycin (c.p.m.)	Without actinomycin (c.p.m.)
1. None	5,080	403,000
2. Pancreatic ribonuclease (24 μg/ml. for 1 h at 37° C in 2 × SSC)	2,437	7,280
3. As in 2 followed by T_1 ribonuclease (0·5 μg/ml. for 30 min at 37° C in 2 × SSC)	2,380	7,100
4. As in 2 followed by heat (3 min at 100° C in 0·1 × SSC), rapid cooling; and again treated with ribonuclease as in 2, in 2 × SSC	1,052	3,800
5. As in 2 followed by heat (3 min at 100° C in 0·1 × SSC), rapid cooling; treated with ribonuclease as in 2, in 2 × SSC, followed by T_1 ribonuclease as in 3	1,020	3,800

The ribonuclease resistant core of the RNA synthesized in the presence of actinomycin co-sedimented with a 4S RNA marker in a sucrose gradient run at 300,000*g* for 50 min. When sedimentation time was increased to 300 min, the core RNA continued to run as a homogeneous peak but with an *s* value of 3·4S relative to marker tRNA (Fig. 1*b*).

Further study of the RNA synthesized in the presence of actinomycin was carried out by molecular sieve chromatography on a column of 'Sephadex *G*-100'. This technique resolves 5S RNA from 4S RNA which sediment together on sucrose density gradients. Burkitt lymphoma cell RNA synthesized in the presence of actinomycin and chromatographed on a 'Sephadex *G*-100' column was resolved into three components (Fig. 2*a*). The higher molecular weight RNA emerged in the void volume trailing behind the first peak of marker ribosomal RNA from rat liver cytoplasm. This portion of the RNA was partially excluded and partially retarded by 'Sephadex *G*-100'. The middle and largest peak co-eluted with the peak of marker 5S RNA (fraction 49, Fig. 2*a*). The last and smallest peak corresponded to the 4S region of the chromatogram. The core RNA eluted with marker 4S RNA from the 'Sephadex *G*-100' column (Fig. 2*b*). Similar profiles were obtained from other cell lines and from the RNA extracted from the liver cytoplasm of actinomycin treated rats. RNA synthesis in the presence of actinomycin has been demonstrated previously in rat liver[3,4]. This RNA contains pseudouridine and is assumed to be tRNA though no amino-acid acceptance has been demonstrated. Because a portion of the RNA synthesized in the presence of actinomycin from cell lines and from rat liver co-eluted with marker tRNA in the present experiment, we examined the methylated base content of the RNA from Burkitt cells to determine if the RNA synthesized in the presence of actinomycin were in part tRNA. Actinomycin treated Burkitt cells were labelled with [^{3}H-methyl] methionine for 40 min and the RNA which had been extracted at 23° C without sodium dodecyl sulphate (SDS) was chromatographed on a 'Sephadex *G*-100' column. Labelled ribosomal RNA is not extracted in these conditions[11]. All labelled material emerged with the marker 4S RNA peak (7,800 c.p.m.), but no acid precipitable labelled material remained after digestion with ribonuclease A. This suggested that the RNA synthesized in the presence of actinomycin contained no methylated bases and was not tRNA. Presumably the observed methyl labelling occurred in relation to the previously synthesized tRNAs. The latter, however, was completely sensitive to ribonuclease digestion, unlike the RNA synthesized in the presence of actinomycin.

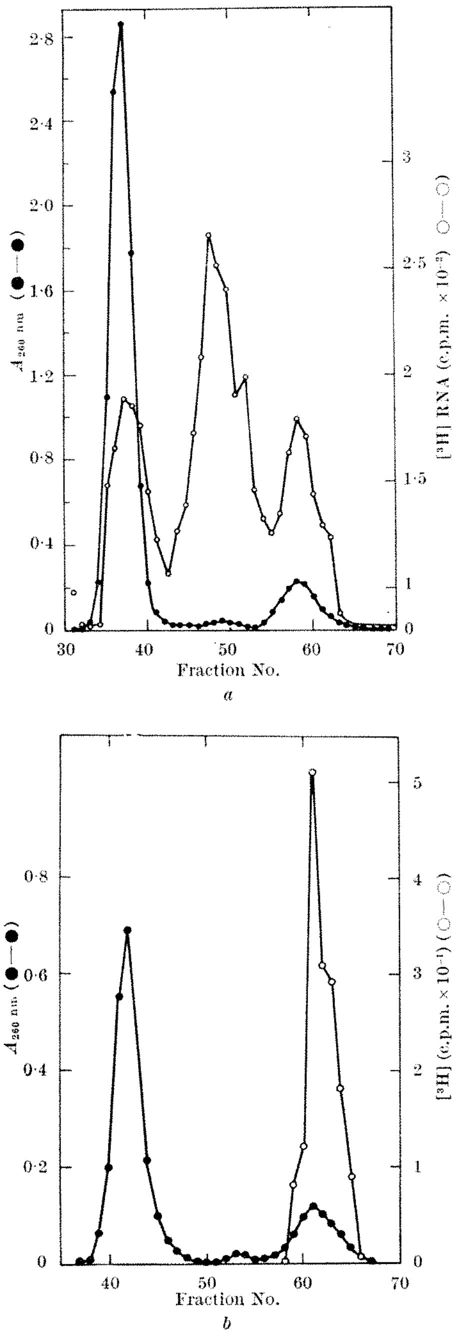

Fig. 2. 'Sephadex *G*-100' chromatography of RNA synthesized in the presence of actinomycin in Burkitt lymphoma cells. RNA was dissolved in 2 × SSC and (*a*) placed on a column together with 0·5 ml. rat liver cytoplasmic RNA containing fifteen absorbance units at 260 nm. In *b* RNA was first digested with ribonuclease A (24 μg/ml. for 30 min at 37° C) followed by incubation with pronase (1·5 mg/ml. for 1 h at 37° C) and placed on a column together with marker rat liver RNA. The column buffer contained 0·2 M NaCl, 0·02 M Tris-HCl (*p*H 7·4), and 2 mM EDTA. 3 ml. fractions were collected. Trichloroacetic acid precipitable ^{3}H-RNA was determined as described in the text.

Further evidence that this RNA was not tRNA was obtained from a study of the rate and extent of hydrolysis by ribonuclease (2·5 μg/ml. at 0° C in 0·2 M NaCl, 0·02 M Tris-HCl buffer, *p*H 7·4, 5 mM $MgCl_2$ at 0° C) of 4S and 5S ^{3}H-labelled RNA from rat liver cytoplasm. After 2 min incubation, 90 per cent of 5S RNA and 38 per cent of the 4S RNA had been hydrolysed. ^{3}H-RNA from Burkitt lymphoma cells synthesized in the presence of actinomycin was degraded by 12 per cent in these conditions. The RNA synthesized in the presence of actinomycin was therefore different from both 5S and most of the 4S RNA of mammalian cells. The distribution of ^{3}H among the nucleotides of the RNA synthesized in the presence of actinomycin following ^{3}H-uridine labelling was examined. After alkaline hydrolysis less than 3 per cent of the label was found in cytidine nucleotides. This ruled out the possibility that the labelling of the 4S component of the RNA synthesized in the presence of actinomycin arises from addition to the 3′ terminus of tRNA.

A series of experiments was performed to demonstrate that RNA was the material containing the radioactive label. The ribonuclease-resistant core of the RNA synthesized in the presence of actinomycin was completely hydrolysed by KOH and was stable to deoxyribonuclease and pronase treatments (Table 3). No label was incorporated into the RNA extracted from actinomycin treated Burkitt cells when ^{3}H-thymidine (20 μCi/ml. for 30 min) was used, thus ruling out a DNA–RNA hybrid RNA. In addition, the RNA synthesized in the presence of actinomycin was found in the aqueous layer following phenol extraction. A DNA–RNA hybrid would have been associated with the interphase or middle layer[10]. RNA was not an artefact of actinomycin treatment because ribonuclease resistant RNA was also obtained from Burkitt cells labelled in the absence of actinomycin. The chromatographic profile of the latter RNA on benzoylated DEAE-cellulose was identical to that of the ribonuclease resistant RNA synthesized in the presence of actinomycin.

Work on RNA bacteriophages has shown that single stranded RNA may be converted to a double stranded form during extraction[20]. The effect of extraction of RNA from Burkitt cells was examined in two ways. To test the effect of phenol deproteinization, actinomycin treated Burkitt cells were lysed with SDS after labelling with ^{3}H-uridine and then deproteinized by passing the material through a silicic acid column[21]. The RNA was then treated with ribonuclease and passed through a benzoylated DEAE-cellulose column. The level of ribonuclease resistance and the chromatographic properties on benzoylated DEAE-cellulose of this material were similar to those of phenol extracted RNA. The effects of detergent and omission of deproteinization were then examined. The supernatant from Burkitt cells, disrupted with a Dounce homogenizer in the absence of detergent, was placed on a sucrose gradient with and without ribonuclease pretreatment. A profile similar to that in Fig. 1*a* was obtained except that the labelled RNA now sedimented more rapidly. The peak of labelled RNA sedimented at 10S and 26S respectively, in the presence and absence of ribonuclease, when deproteinization was omitted. This suggested that either the RNA synthesized in the presence of actinomycin is of larger *s* value in the intact cell and is partially degraded by extraction, or that proteins are closely associated with this RNA in the cell.

The ribonuclease resistant core of the RNA synthesized in the presence of actinomycin had several properties of a double-stranded RNA. When this RNA was dissolved in $0{\cdot}1\times$SSC instead of $2\times$SSC (SSC = 0·15 M NaCl–0·015 M sodium citrate, *p*H 7·0) there was a loss of all detectable labelled acid insoluble material on further ribonuclease treatment (Table 2). Such stability to ribonuclease at high ionic strength and susceptibility at low ionic strength has been used as a criterion for a double-stranded RNA[20–25]. On columns of benzoylated DEAE-cellulose[14,26] which resolves mammalian RNAs on the basis of secondary structure during elution on a gradient of NaCl[15], the ribonuclease resistant core eluted with a synthetic homopolymer duplex poly I·poly C. This RNA also elutes with the entirely base paired replicative form of the RNA virus, Semliki Forest virus (unpublished data). This suggested that the ribonuclease resistant core of the RNA synthesized in the presence of actinomycin is an entirely base paired duplex.

The melting profile of the ribonuclease resistant core RNA was determined by assaying ribonuclease sensitivity of preparations heated to different temperatures. A transition was observed at 70° C, which was compatible with a highly structured RNA[27].

Table 4. BASE COMPOSITION OF CHICK EMBRYO FIBROBLAST ^{32}P-RNA SYNTHESIZED IN THE PRESENCE OF ACTINOMYCIN D AND RESISTANT TO HYDROLYSIS BY PANCREATIC RIBONUCLEASE

Base	Moles per cent
Uridylic acid	23·3
Guanylic acid	30·8
Cytidylic acid	23·9
Adenylic acid	22·0

In spite of these findings the base composition of the RNA is incompatible with an entirely base paired structure. Base composition studies revealed that the ribonuclease resistant core RNA from chick embryo fibroblasts had a high guanine content (Table 4). Additional observations suggested that this RNA had unusual features and was not a simple base paired structure. Heating to 100° C and rapid cooling disrupt double stranded RNAs, making them entirely sensitive to pancreatic ribonuclease. Treatment with dimethyl sulphoxide (DMSO) followed by dialysis[28] has the same effect. But after these treatments, only 60 per cent of the ribonuclease resistant core became sensitive to ribonuclease; 40 per cent of the material remained acid precipitable. This residual acid precipitable RNA can also be observed at temperatures above the T_m.

Ribonuclease A treatment may be expected to generate nuclease resistant oligomers with the general formula (purine)$_n$ pyrimidine. Such fragments would have been susceptible to further digestion with T_1 ribonuclease, but no change in acid precipitable material was observed following this treatment (Table 3, expt. 3). This indicates that the core RNA was not a large (purine)$_n$ pyrimidine oligonucleotide. Another structure which can be invoked from the base composition is a base paired molecule associated with a pG rich polymer. Heating to 100° C, rapid cooling, followed by ribonuclease digestion would have left such a proposed pG rich strand largely intact and would have accounted for the residual acid precipitable RNA observed. This possibility is ruled out because the RNA is not susceptible to T_1 ribonuclease digestion. Table 3, experiment 5, demonstrated that the 40 per cent residual RNA remains intact after T_1 treatment. It is possible that a collection of pG rich fragments could aggregate and behave like large oligonucleotides with a sufficiently high degree of secondary structure to resist melting and T_1 digestion[29]. But when the RNA synthesized in the presence of actinomycin was treated with T_1 ribonuclease either before or simultaneously with exposure to pancreatic ribonuclease, no difference was observed in the resulting level of ribonuclease resistant material.

Unusual Form of RNA

The evidence suggests a species of RNA in animal cells which is synthesized in the presence of actinomycin D. It is 40 per cent resistant to hydrolysis by ribonucleases A and T_1, a smaller portion also resistant to hydrolysis following heat and DMSO denaturation. No single explanation exists for all these findings. Among the many possibilities is that the RNA is transcribed from a protected or actinomycin resistant region of the DNA genome, or transcribed within a cell structure impermeable to actinomycin[30]. Alternatively, the RNA may be synthesized on an RNA template. A latent RNA virus in cell lines could be invoked, but this would suggest that latent RNA viruses are ubiquitous in animal cells. The RNA may represent replication of an RNA plasmid, but such a mode of synthesis has never been observed in animal cells.

Many actinomycin resistant reactions in mammalian cells result in ribonucleosides being converted to acid-insoluble forms, including polyadenylate synthesis[31] and chain extension of pre-existing polynucleotides[32–38]. These reactions have not been ruled out entirely as the basis for the present observations, but the products differ from the RNA being discussed in base composition and resistance to ribonuclease.

The ribonuclease-resistant core of the RNA synthesized in the presence of actinomycin could be attributed to either a high degree of secondary structure or to covalently bound groups and base substitutions. Bound amino-acids or peptides, and intermolecular disulphide bridges[37], could explain the small portion of RNA resistant to ribonuclease digestion following heat and DMSO denaturation. The increased *s* value of this RNA when deproteinization was omitted from the extraction procedure is support for this hypothesis. The RNA associated with nucleohistones[38] has many features in common with the present RNA, including partial resistance to ribonuclease.

Colby and Duesberg[23] have reported that vaccinia virus infection induces synthesis in chick embryo cells of a double-stranded RNA. This material has a thermal denaturation curve and equilibrium sedimentation characteristics of a double-stranded molecule. It also exhibits the difference in ribonuclease sensitivity in high and low salt typical of double-stranded molecules. No base composition data are reported. A smaller amount of partially ribonuclease-resistant RNA which did not hybridize with the vaccinia genome is present in uninfected chick cells. This material may be similar to the RNA reported here.

An understanding of the RNA synthesized in the presence of actinomycin has practical application in the examination of the product of RNA virus synthesis in animal cells. Replication of RNA viruses is studied routinely by inhibiting host RNA synthesis with high concentrations (1–5 μg/ml.) of actinomycin. The assumption is made that all subsequent RNA synthesis is viral specific. But, as shown by our experiments, a low level of host RNA synthesis persists. It cannot be assumed that all RNA fractions obtained from the infected host are, in fact, specified by the virus.

In conclusion, the assumption that the synthesis of all informational RNA in normal mammalian cells is directed by DNA templates must be held in abeyance until the origin of the present RNA has been determined.

Cultures, Extractions and Determinations

The methods used in preparing monolayers of primary chick fibroblasts and pools of the Semliki Forest virus for the preparation of the replicative form of the virus have been previously described[8]. Collection, purification and culture conditions for resting and phytohaemagglutinin stimulated human peripheral lymphocytes were described by Cooper[9]. Culture conditions for the Burkitt lymphoma cells were the same as those for normal lymphocytes except for the use of medium RPMI 1640 instead of Eagle's minimal essential medium. Lymphoid cells were preincubated with actinomycin (Merck, Sharp, and Dohme) at 5 μg/ml. for 30 min; other cell lines, at 2·5 μg/ml. All cells in suspension cultures were concentrated five-fold before the addition of ^{3}H-uridine, 20 μCi/ml. or [^{3}H-methyl]methionine, 20 μCi/ml. (New England Nuclear). For the latter, in RNA methylation experiments, cells were incubated in medium containing 0·05 the usual concentration of amino-acids plus 1 mM sodium formate. ^{32}P labelling experiments were performed with chick embryo fibroblasts. The monolayers were preincubated in low phosphate containing medium with 2 per cent serum for 30 min. Actinomycin was added for 30 min followed by carrier free ^{32}P-phosphoric acid (New England Nuclear), 0·2 mCi/plate, for an additional 40 min.

RNA was extracted[10,11] at 37° C in the presence of bentonite and SDS. Unlabelled cytoplasmic RNA from rat liver[12] was added to the final aqueous phase of the extraction procedure. RNA was washed twice and dissolved in 2 × SSC. When ribonuclease digestion was performed, samples were also treated with pronase (Calbiochem) (1·5 mg/ml. for 1 h at 37° C) to inactivate the ribonuclease[13].

Chromatography on the benzoylated DEAE–cellulose[14] (Schwarz BioResearch) column has been described[15]. 'Sephadex *G*-100' column (1·5 cm × 90 cm) chromatography was performed using a buffer containing 0·2 M NaCl, 0·02 M Tris-HCl (*p*H 7·4), 2 mM EDTA. Sucrose density gradients were analysed and the relative *s* values were calculated by previously described methods[16,17].

For the base composition determination of ^{32}P-RNA from chick embryo fibroblasts, the RNA resistant to ribonuclease A and T_1 (Worthington Biochemical) was passed through a benzoylated DEAE-cellulose column. The single peak of ^{32}P-RNA co-eluting with the synthetic homopolymer duplex poly I·poly C (Miles Laboratories) was hydrolysed for base analysis[18].

Tissues from rats following intraperitoneal injections of actinomycin and ^{3}H-orotic acid (Schwarz BioResearch) were obtained using the protocol of Revel and Hiatt[3].

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Origin of the Alpha Rhythm

by

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The 8–12 Hz rhythmical waveform that can be recorded from the scalp of most human subjects when their eyes are closed does not originate in brain tissue. It is generated by physiological tremor in the extra-ocular muscles modulating the field set up by the standing potential of the eye.

SINCE the regular potential fluctuations that can be recorded from the human scalp were first described[1], the generally accepted view has been that the waves originate mainly in the occipital cortical neurones. The cells are pictured as beating in unison at a frequency of about 10 Hz whenever the cortex is "resting", that is, not receiving an input of patterned vision[2,3].

There are, however, a number of rather discordant features about the hypothesis. No convincing evidence has yet shown any "physiological function" for this alpha rhythm; 2–5 per cent of people never exhibit the waves and yet seem quite normal. In spite of the large amplitude of the waves (50 to 150 μV) and their widespread distribution over the head, there are no perceptual or motor concomitants that accompany their presence or absence. This is surprising in view of the much smaller size, on the scalp, of the evoked response (2–5 μV) which is presumably the electrical sign of perceptual activity within the grey matter. It is also disconcerting to find that the electrical indication of the onset of "rest" in the cortex is usually a ten-fold increase in the amplitude of a waveform.

There are phase-reversal points of the waves at the occipital regions of the skull, and their amplitude is at a maximum there; the site of origin was therefore postulated[4] to be in a dipole in the grey matter of each occipital lobe. This view is inconsistent, for it is well established that alpha waves in the frontal region, farther away from the supposed site of origin and smaller in amplitude, tend to lead the occipital waves by a variable interval, usually about 5 ms.

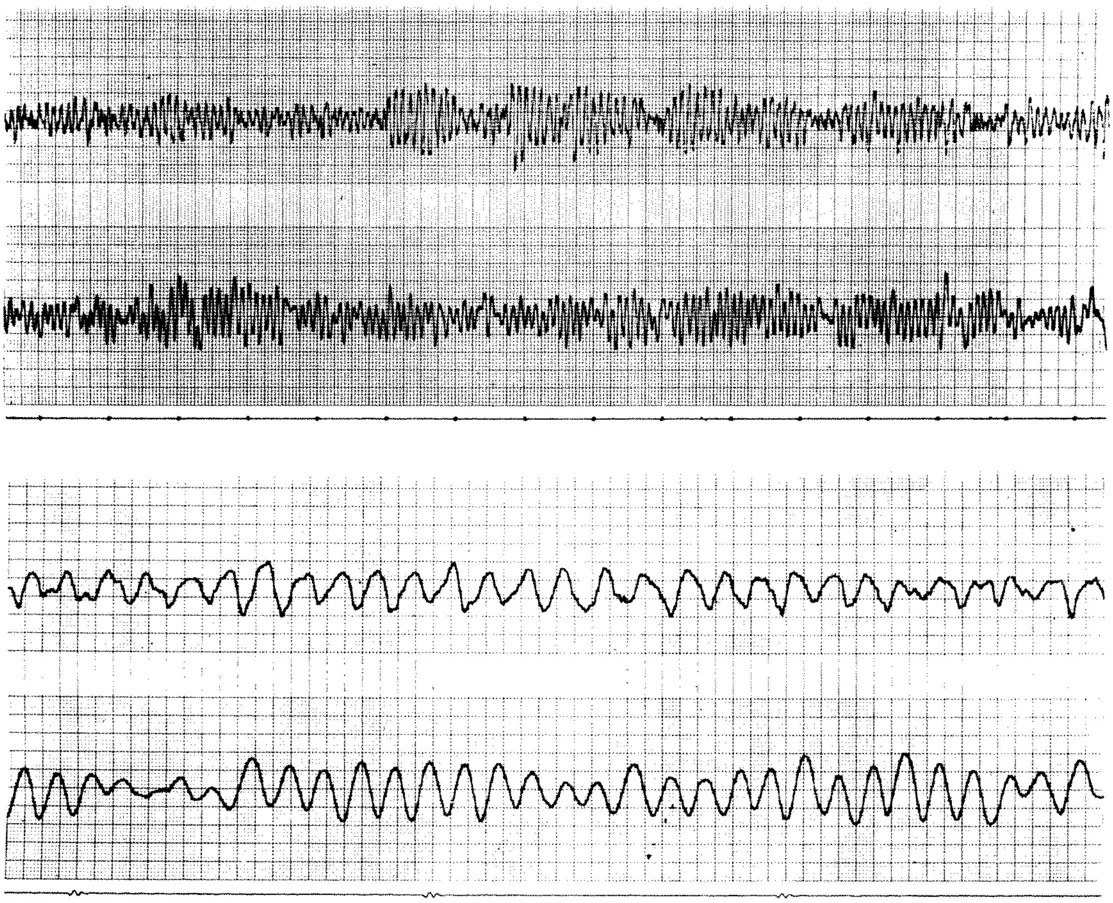

Fig. 1. Comparison between alpha waves recorded simultaneously with tremor waves from the forefinger, in a normal subject. Alpha waves were recorded as described by Lippold[12] and tremor by using a beam of light and phototransistor. Calibration per large square; alpha waves, 10 μV; tremor waves, 0·2 mm. Time, 1 s. Note, in the top pair of records, the similarity of general waveform (waxing and waning, and so on) and, in the bottom pair, the detailed similarity between individual waves.

New Explanation for Alpha Rhythm

The following alternative has been proposed[10]. The extra-ocular muscles, in common with all other skeletal muscles, have feed-back control; in common with other muscles, oscillation will take place at about 10 Hz in this servo when its loop gain exceeds unity[5]. In most people this condition tends to occur when the eyes are closed, or when there is no sharp image on the retina for the eye to be fixated on by feedback from the retina and the visual cortex.

The source of current for generating the waveform of alpha rhythm is the standing potential across the eye. It is conducted back into the interior of the cranium by the extra-ocular muscles which act as variable shunts producing an alternating 10 Hz field on the surface of the scalp whenever these muscles show physiological tremor.

The electrical record of alpha rhythm from the head closely resembles the mechanical record of the tremor of a limb (compare refs. 6 and 7). When both are recorded simultaneously from the same subject, they seem in general to correspond well; for example, the records look alike in response to loud sounds during sleep or anaesthesia[8]. Clearly, no constant phase relation exists between the two types of wave, nor is their frequency exactly the same[8] although the frequency spectrum has a similar form (see also Fig. 1). The frequency of tremor is slow in small children, gradually rising until the normal 10 Hz is reached in early adult life; the frequency of alpha waves has a similar variation with age[6,7]. In Parkinson's disease, tremor in muscle is characteristically at about 5/s. If the disease involves the eye muscles the predominant cortical rhythm is often at 5/s[9]. Further, subjects with both eyes enucleated have no alpha rhythm[2], although it must be stated that cortical blindness also leads to its disappearance (and subsequent reappearance if the sight is restored).

Anyone who has prominent alpha rhythm can make a simple observation to show that eye tremor can occur. A bright point-source of light is viewed against a completely dark field. If the gaze is now swept at a suitable speed (from top to bottom of the field of view in 1 s), vertically past the source, the resulting after-image can be examined. It will be found to be an approximately sinusoidal trace.

It has already been shown that local cooling of a limb slows its tremor because the delay in the servo-loop is then increased, as a result of lengthened muscle twitch time[5]. Cooling the orbit in man has a similar effect on the frequency of alpha rhythm[10], a finding which indicates that a similar servo-system could be in operation for the eye muscles.

Varying the Corneo-retinal Potential

Alteration of the illumination of the eye changes the value of this potential by a factor of up to three[11]. If alpha waves are indeed generated by the potential across the eye, the amplitudes of the former and the latter should co-vary.

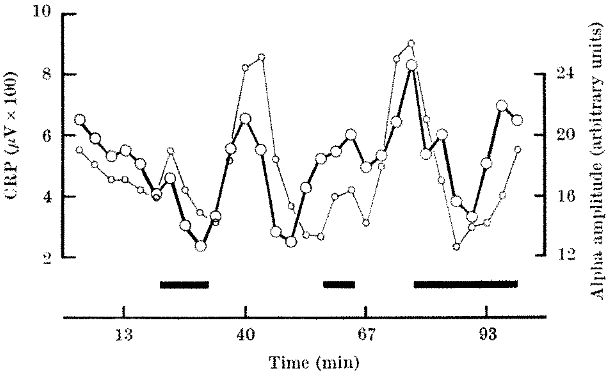

Fig. 2. Graph showing corneo-retinal potential (CRP, thin line) plotted with alpha amplitude (thick line) during the course of an experiment in which the former was varied experimentally by altering the intensity of ambient illumination. The CRP was measured by recording from electrodes in the inner and outer canthi while the gaze was rapidly switched between two fiducial marks by the subject (see ref. 11). Alpha amplitude was measured from occipital leads (O_2 to T_6 in the standard 10–20 system) using an Ediswan wave analyser. Black bars indicate that the subject was in darkness; at other times an X-ray viewing screen provided diffuse illumination. Note that the full range of CRP values occurs in both light and dark conditions; the results cannot thus be explained in terms of an effect of ambient illumination on retinal or cortical function. The alpha wave recordings were taken in all cases with eyes closed; 10 s periods were integrated. ($r = 0·55$; $t = 3·5$; $P < 0·01$.)

Fig. 2 shows the results of an experiment in which the value of the corneo-retinal potential was varied (thin line; small circles). Alpha wave amplitude was measured with an Ediswan analyser. There is an excellent correspondence between the magnitude of the corneo-retinal potential and the amplitude of the alpha waves ($r = 0·55$; $t = 3·5$; $P < 0·01$).

This experiment is, however, unsatisfactory because alpha rhythm tends to change in amount from time to time during a long experiment, as a result of the operation of factors such as attention, drowsiness or eye movements. (It is interesting to note that, if account is taken of this and mean amplitude per wave is computed, a better correlation can be obtained; for example, $r = 0·9$; $P < 0·0001$). The mean amplitude of alpha waves on the two sides of the head can be compared by a method I have already described[12]. The ratio of the alpha amplitude on each side can be compared with the ratio of the values of the corneo-retinal potential as the latter is experimentally varied. Fig. 3 gives the results of an experiment of this kind showing that the alpha amplitude depends, with a high degree of significance, on the value of the corneo-retinal potential on the same side of the head. This experimental design eliminates the difficulties (attention, drowsiness and so on) mentioned here, for they affect both sides similarly, and moreover it shows that the effect described cannot be mediated by nervous connexions from the retina because each visual cortex receives an equal innervation from the two eyes.

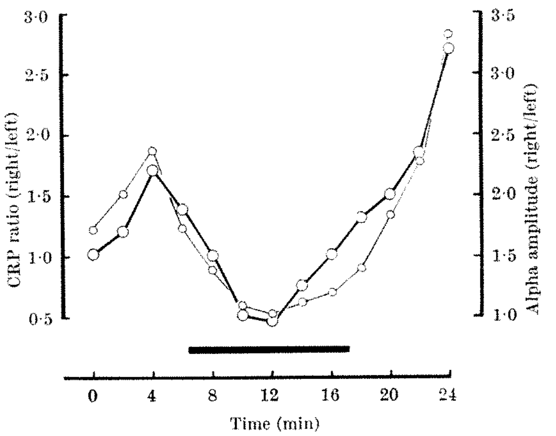

Fig. 3. Comparison between two sides of the head. The ratios between alpha amplitudes (thick line) on each side and CRP values (thin line) on each side are plotted. (Amplifiers, Tektronix type 122, T.C. = 0·02 s; H.F. cut at 50 Hz. Sanborn type 320 pen recorder.) Alpha amplitude measured by modification of method of Walter and Yeager[13] (area enclosed by peaks of waves, recorded at 20 mm/s, measured with a planimeter. Values are means of four periods of 5 s each). CRP measured for each eye separately as described in legend to Fig. 2. Illumination of each eye was altered independently by using light-tight goggles. Dark bar indicates time during which right eye was dark and left illuminated. At all other times left eye was dark and right illuminated. If values obtained on left side of the head only are plotted, they show that the intercept of the regression of y on x cuts the x-axis approximately at the value found for EEG amplitude with the eyes open.

When the corneo-retinal potential values are plotted against the alpha amplitude values for one side of the head only, the regression line cuts the alpha amplitude axis at a value that approximates to the mean EEG amplitude found when the eyes are open with fixated gaze. This indicates that the waveform of the EEG obtained with the eyes open does not originate from the corneo-retinal potential. It may represent either true cortical electrical activity, or it may be the attenuated electromyogram of other muscles in the head and neck region.

From the results of this very simple experiment, it can be concluded that occipital alpha waves are generated by the standing potential across the eyes. Other evidence shows that the likely mechanism involves some form of modulation of this potential by oscillation in the extra-ocular muscles (tremor) at about 10 Hz when there is no sharply focused image on the retina.

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A Paradox in the Interaction of the Gravitational and Electromagnetic Fields?

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The assumption that the extent of the interaction of the electromagnetic and gravitational fields is fundamentally dependent on the frequency of the electromagnetic field is consistent with data on the deflexion of radiation by massive bodies.

RECENT experiments designed to test some consequences of the general theory of relativity have hinted that the theory may not be adequate to explain all the pertinent observations. Sadeh and others have found, for example, that the interaction between electromagnetic and gravitational fields departs markedly from the predicted behaviour. Further, all measurements of the deflexion of light by the Sun's gravitational field have given larger deflexions than expected. One fundamental assumption and two subsidiary requirements apparently suffice to account for the anomalous experimental results.

The fundamental supposition is that the extent of the interaction of the electromagnetic field with the gravitational field is functionally dependent on the frequency of the electromagnetic field. Two subsidiary requirements will be introduced at appropriate stages in the following discussion. It should be noted, however, that the stated assumption is not consistent with the general theory of relativity, which requires that the extent of the interaction of gravitational and electromagnetic fields be independent of frequency.

The chief manifestation of the interaction of gravitational and electromagnetic fields is the decrease of the velocity of light relative to Euclidean space–time coordinates. According to the general theory, the decrease is given by

$$c^2 = c_0^2\left(1 + \frac{2\Phi}{c_0^2}\right)$$

where c is the velocity of light in the gravitational field, c_0 that in field-free space, and Φ the potential of the gravitational field[1]. This formula is independent of the frequency of the light, and according to our fundamental assumption not fully correct. We postulate that c is linearly dependent on c_0; that is

$$c = c_0(1 + \alpha)$$

where α is some dimensionless function of ν and Φ; that is,

$$\alpha = f(\nu, \Phi)$$

On invoking the general theory as an approximate boundary condition, it follows that

$$\alpha = \frac{\beta(\nu)\Phi}{c_0^2}$$

where $\beta(\nu)$ is a function determined by the experimental data from the deflexion of light in the Sun's gravitational field and those of Sadeh *et al.*[2,3]. Thus

$$c = c_0\left(1 + \frac{\beta(\nu)\Phi}{c_0^2}\right)$$

Using this expression for c, we can derive the deflexion of a ray of light passing close to a massive body, as Einstein did in his 1911 paper[4]; we find that the deflexion δ is

$$\delta = \frac{2\beta(\nu)GM}{c_0^2 r_0} \tag{1}$$

where G is the constant of gravitation, M the mass of the (arbitrary) massive body, and r_0 the perihelion distance of the path of the light ray. For $\beta(\nu) = 2$, the above expression reduces to the δ predicted by the general theory. It is clear from our expression for δ that electromagnetic radiation of different frequencies will be deflected by different amounts in a gravitational field. This result, if true, provides a means of distinguishing between a gravitational field and an accelerated frame of reference. For suppose we observe the paths of two initially coincident light rays of different frequencies. If the paths of both rays remain the same, we have an accelerated frame of reference; whereas if they diverge, we have a gravitational field. Thus we reluctantly conclude that if the electromagnetic–gravitational interaction is frequency dependent, then the equivalence principle cannot be valid.

We now examine the experimental data provided by the various eclipse expeditions to see whether the assumption of the frequency dependence of the electromagnetic–gravitational field interaction is warranted. If the observed deflexion corresponds to that predicted by the general theory, then $\beta(\nu) = 2$, and the assumption of frequency dependence is unnecessary and unjustified. But if the observed deflexion is not the same as the prediction, then $\beta(\nu) \neq 2$, and the assumption of frequency dependence is necessary and justified.

Some of the results from the eclipse expeditions are very unreliable. In particular, the results of the 1936 and 1952 expeditions are virtually worthless for two reasons. First, in both cases almost all the stars in the star-field whose positions were measurable were more than three solar radii from the Sun. This means that the displacements were barely measurable and had large errors. The extrapolation from these measurements to a limb deflexion has a built-in error so large as to make the results completely unreliable. Second, in both cases there were serious technical deficiencies which serve to make the results even more untrustworthy. On the other hand, the results of the 1919, 1922, 1929 and 1947 expeditions are reliable and their errors not unreasonable.

Mikhailov has calculated the limb deflexion for all the eclipses, using a standard method of reduction of the data[5]. Because his method is more accurate in some cases than the method used by the original authors, we shall use his results exclusively. When one averages the results and the probable errors one obtains for the observed limb deflexion $\delta = 2{\cdot}02 \pm 0{\cdot}17''$. The prediction of the general theory does not even approach the lower limit of the probable error, so we conclude that $\beta(\nu) > 2$, and the assumption of frequency dependence is justified.

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It is often claimed that the measurement of δ is based only on several observations and is therefore unreliable. This statement is misleading. It is true that only four eclipses have produced usable plates, but the determination of δ is based on the measurements of displacements of hundreds of stars and not just a few as the statement implies.

With one more subsidiary requirement (or boundary condition) a tentative explicit expression for $\beta(\nu)$ may be determined in the following manner. We stipulate: As ν goes to infinity, $\beta(\nu)$ goes to unity, or

$$\lim_{\nu \to \infty} \beta(\nu) = 1$$

This requirement may be reformulated in terms of the deflexion as

$$\lim_{\lambda \to 0} \delta = \frac{2GM}{c_0^2 r_0}$$

Analysis of the experimental data shows that for small values of the wavelength λ, δ is approximately proportional to $\lambda^{1/2}$. Thus we posit

$$\delta^2 = \left(\frac{2GM}{c_0^2 r_0}\right)^2 + K(r_0)\lambda + L(r_0)\lambda^2 \qquad (2)$$

where the coefficients $K(r_0)$ and $L(r_0)$ are functions solely of r_0. When $K(r_0)$ and $L(r_0)$ are determined (r_0 equal to the Sun's radius) from all the existing data (especially those of Sadeh *et al.*) that will be presented later on, we obtain

$$K(r_0) = 1{\cdot}65 \times 10^{-6}\ \text{cm}^{-1}$$
$$L(r_0) = 1{\cdot}75 \times 10^{-14}\ \text{cm}^{-2}$$

$\beta(\nu)$ may now be computed from equations (1) and (2), which gives

$$\beta^2(\nu) = \left(\frac{c_0^2 r_0}{2GM}\right)^2 (K(r_0)\lambda + L(r_0)\lambda^2) + 1 \qquad (3)$$

Using this relation for $\beta(\nu)$ and $\lambda = 4800$ Å (the average of the wavelength of maximum response for the photographic plates used), we obtain a value of δ which agrees with observation.

We now consider another test involving the interaction of the electromagnetic and gravitational fields. If the time taken for radiation to travel from one point to another changes as a function of time because of intervening gravitational fields, there will be a change in the frequency of the radiation such that

$$\frac{\Delta\nu}{\nu} = -\frac{\mathrm{d}\Delta\tau}{\mathrm{d}t}$$

where $\Delta\tau$ is the increased time of travel from the source to the observer. There are two factors that determine $\Delta\tau$: the path length and the velocity along the path. Consider the case of the electromagnetic radiation of a star passing close to the Sun. If either the general theory or our contention is correct, a time delay will occur, because both the path and the velocity of electromagnetic radiation are dependent on the gravitational fields present. There is, however, a significant distinction. The fractional frequency shift ($\Delta\nu/\nu$) will, according to the general theory, be the same for all frequencies and numerically very small, whereas on our assumption it will depend on the frequency of the radiation, because both path and velocity are dependent on frequency in a gravitational field. The predictions do differ markedly for long wavelengths, and we shall now derive the frequency shift supposing the interaction of the electromagnetic and gravitational fields to be dependent on frequency.

It follows from special relativity theory that, for a light ray,

$$\mathrm{d}r^2 + r^2\mathrm{d}\varphi^2 - c^2\mathrm{d}t^2 = \mathrm{d}s^2 - c^2\mathrm{d}t^2 = 0$$

that is,

$$\tau = \int \frac{\mathrm{d}s}{c} = \frac{1}{c_0}\int \frac{\mathrm{d}s}{(1+\alpha)}$$

Hence

$$\begin{aligned}\Delta\tau &\approx \frac{1}{c_0}\int (1+\alpha)\mathrm{d}s - \frac{s_0}{c_0} \\ &= \frac{1}{c_0}(s-s_0) + \frac{\beta(\nu)GM}{c_0^3}\int \frac{\mathrm{d}s}{r} \qquad (4)\end{aligned}$$

where s_0 is the straight line from the source to the observer, s is the total path length, and ν is assumed to be sufficiently large to allow the approximation of c by c_0 in the $(1+\alpha)$ term. Equation (4) can be rewritten as

$$\begin{aligned}\Delta\tau &= \frac{1}{c_0}(s-s_0) + \frac{\beta(\nu)GM}{c_0^3}\int_{\theta_{\text{source}}}^{\theta_{\text{observer}}} \sec\theta\ \mathrm{d}\theta \\ &= \frac{1}{c_0}(s-s_0) + \frac{\beta(\nu)GM}{c_0^3}\Big[\ln(\sec\theta + \tan\theta)\Big]_{\theta_s}^{\theta_{\text{ob}}}\end{aligned}$$

where θ is the angle between the radius vector from the Sun's centre to the perihelion point on the path and the radius vector to a point on the path. We define

$$R_{\text{ob}} = \sqrt{r_{\text{ob}}^2 - r_0^2}$$

and

$$R_{\text{s}} = \sqrt{r_{\text{s}}^2 - r_0^2}$$

Then

$$\Delta\tau = \frac{1}{c_0}(s-s_0) + \frac{\beta(\nu)GM}{c_0^3}\left[\ln\left(\frac{r_{\text{ob}} + R_{\text{ob}}}{r_0}\right) + \ln\left(\frac{r_{\text{s}} + R_{\text{s}}}{r_0}\right)\right]$$

If $r_{\text{s}} \simeq R_{\text{s}}$ and $r_{\text{ob}} \simeq R_{\text{ob}}$, we get

$$\Delta\tau = \frac{1}{c_0}(s-s_0) + \frac{\beta(\nu)GM}{c_0^3}\ln\left(\frac{4r_{\text{ob}}r_{\text{s}}}{r_0^2}\right)$$

Further, it is easy to show that

$$s \simeq s_0 + r_{\text{e}}\left(\frac{1}{\cos\delta} - 1\right)$$

where r_{e} is the distance from the perihelion point of the path to the observer. As a result,

$$\Delta\tau \simeq \frac{r_{\text{e}}}{c_0}\left(\frac{1}{\cos\delta} - 1\right) + \frac{\beta(\nu)GM}{c_0^3}\ln\left(\frac{4r_{\text{ob}}r_{\text{s}}}{r_0^2}\right)$$

Differentiating this equation with respect to time, we arrive at

$$\frac{\mathrm{d}\Delta\tau}{\mathrm{d}t} = -\frac{r_{\text{e}}}{c_0}\left(\frac{\sin\delta\ \dfrac{\mathrm{d}\delta}{\mathrm{d}t}}{\cos^2\delta}\right) - \frac{2\beta(\nu)GM}{c_0^3 r_0}\ \frac{\mathrm{d}r_0}{\mathrm{d}t}$$

Because in our case we are interested in $\delta < 1°$, $\sin\delta \simeq \delta$, and $\cos^2\delta \simeq 1$, this reduces to

$$\frac{\Delta\nu}{\nu} = -\frac{\mathrm{d}\Delta\tau}{\mathrm{d}t} = \frac{\delta r_{\mathrm{e}}}{c_0}\frac{\mathrm{d}\delta}{\mathrm{d}t} + \frac{\delta}{c_0}\frac{\mathrm{d}r_0}{\mathrm{d}t}$$

But

$$\frac{\mathrm{d}\delta}{\mathrm{d}t} = \frac{\delta}{r_0}\frac{\mathrm{d}r_0}{\mathrm{d}t}$$

so

$$\frac{\Delta\nu}{\nu} = -\frac{\mathrm{d}\Delta\tau}{\mathrm{d}t} = \frac{\delta}{c_0}\frac{\mathrm{d}r_0}{\mathrm{d}t} + \frac{r_{\mathrm{e}}\delta^2}{r_0 c_0}\frac{\mathrm{d}r_0}{\mathrm{d}t}$$

The term in δ^2 represents the change in ν caused by the change in time lag resulting from the increase in path length, and the term in δ represents the change in ν resulting from the velocity variation along the path. This result differs from that of the general theory chiefly because it treats $\beta(\nu)$ as a function of ν, while in the general theory $\beta = 2$ for all frequencies.

We now consider an experiment carried out by Sadeh *et al.*[2], where the apparent frequency shift for the 21 cm absorption line of Taurus A was determined when it passed close to the Sun on June 15, 1967. (The experiment was repeated in 1968 and yielded essentially the same results as those described below.) According to the general theory,

$$\frac{\Delta\nu}{\nu} = \frac{4GM}{c_0^3 r_0}\frac{\mathrm{d}r_0}{\mathrm{d}t}$$

leading to a prediction of $\Delta\nu$ for the 21 cm line that is three orders of magnitude smaller than that observed.

To determine the prediction based on our fundamental assumption, we first determine $\beta(\nu)$ for $\lambda = 21$ cm from equation (3) by substituting the Sun's radius for r_0 and the previously given values of $K(r_0)$ and $L(r_0)$. The outcome is $\beta(\nu) = 1{\cdot}39 \times 10^3$. The maximum value of $\Delta\nu$ may be computed using this value of $\beta(\nu)$, $r_0 = 3{\cdot}48 \times 10^{11}$ cm (which corresponds to the angular distance 1·25° from the Sun's centre), and $\mathrm{d}r_0/\mathrm{d}t = 2{\cdot}04 \times 10^6$ cm s^{-1}, giving $\Delta\nu_{\max} = 121$ Hz. This is in good agreement with the observed maximum value of about 150 Hz.

Another recent experiment that supports the assumption of the frequency dependence of the electromagnetic–gravitational interaction has been performed by Sadeh *et al.*[3]. They determined the relative rate of two caesium clocks as one of the two clocks was moved in successive steps from North Carolina to Nova Scotia. These authors demonstrate that no current theory can explain the observed frequency shift.

In our context it is worth considering the gravitational redshift that arises from the oblateness of the Earth. The expression for this effect is

$$\frac{\Delta\nu}{\nu} = \frac{GM}{c^2}\left(\frac{\Delta r}{\bar{r}^2}\right)$$

where $\bar{r}$ is the average of the distances from the centre of the Earth to two points on its surface and Δr is the difference between these distances. The approximate values of $\bar{r}$ and Δr corresponding to the maximum value of $\Delta\nu/\nu$ observed are $6{\cdot}37 \times 10^8$ cm and $2{\cdot}5 \times 10^5$ cm respectively. Positing that c is roughly the same as c_0 (as suggested by the general theory) and computing $\Delta\nu/\nu$, we obtain a value about one order of magnitude smaller than the observed value. But the assumption $c \simeq c_0$ for $\nu \simeq 1$ Hz (the condition realized in the experiment) is fallacious. Instead, we must use

$$c = c_0\left(1 + \frac{\beta(\nu)\Phi}{c^2}\right)$$

When $\beta(\nu)/c^2$ is computed for $\nu = 1$ Hz, it is seen to be equal to $1{\cdot}10 \times 10^{-11}$ s^2 cm^{-2}. Substituting this value, along with the values of other required quantities, in the equation for $\Delta\nu/\nu$, we arrive at $\Delta\nu/\nu \simeq 3 \times 10^{-12}$, which is in good agreement with the value experimentally obtained by Sadeh.

Sadeh *et al.*[6] have continued a search for novel effects capable of changing the frequency of a spectral line by measuring the times of arrival of pulses from pulsar CP 0950. According to a hypothesis advanced earlier by Sadeh, a cumulative delay of the pulse arrival time should have appeared as the line of sight to CP 0950 passed close to the Sun. A transient retardation was indeed observed, but no cumulative retardation, contrary to Sadeh's prediction.

On brief reflexion it becomes clear that our conjecture predicts a transient retardation, similar to the prediction of the general theory, but larger. No cumulative effect, however, is predicted. Hence our theory agrees with the experimental results.

Markowitz[7] attempted to determine whether the frequency of a radio wave was decreased, according to the hypothesis of Sadeh, as it passed through the Earth's gravitational field. The crucial aspect of this experiment is that the determination is independent of the rates of the clocks used to observe the frequency of the wave. The prediction of our theory is that the relative rates of the clocks would be different because of the previously described gravitational effect, but that the frequency of the signal carrier-wave itself would merely be negligibly redshifted because of its high frequency (100 KHz). Markowitz's results, which showed that the carrier-wave is only minutely shifted, agree with our theory. His findings, however, do not invalidate Sadeh's results, because the relative rates of the clocks as a function of the local gravitational field were not examined at all. We therefore infer that Markowitz's data preclude the validity of Sadeh's conjecture, but are not at variance with our theory.

Warner and Nather[8], considering the time-delay between pulses in the yellow and ultraviolet from NP 0532, have reached the conclusion that the speed of light is independent of frequency. There are, however, several variable stars[9] which have time-delays of the order of 10^2 s. We therefore feel that it would be premature to reject the frequency dependence theory on the basis of Warner and Nather's results.

It should be noted that our calculations have used a Euclidean metric. The reason is that if one adheres to a geometrical representation of the gravitational field, the frequency dependence requires the introduction of an infinite number of superimposed space–times of varying metrics. Physically, this interpretation is somewhat less than desirable. It is possible, however, to use the Schwarzschild metric, in which case the deflexion and fractional frequency shift equations must be multiplied by a factor of two and the values of the coefficients $K(r_0)$ and $L(r_0)$ must be suitably adjusted to fit the experimental data. But the choice of metric has no bearing on any of our conclusions, because no single frequency-independent metric can be used as a basis for explaining all the experiments we have discussed.

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The Nature of Pulsar Radiation

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Pulsar radiation may be due to particles which gyrate while moving in bunches along curved orbits. If emitted by electrons, the pulses should come from near the speed of light circle; if emitted by protons, they should arise within a few stellar radii of the underlying star.

THE amount of energy released by a neutron star in the form of pulsed radiation seems to be a small fraction of the total loss of rotational energy. An understanding of the radiation mechanism, however, would provide information about the magnetosphere of the neutron star and about general pulsar electrodynamics. Our purpose here is to discuss the nature of the radiation and to give some estimates for the density, energy and amount of correlation in the emitting plasma.

We find that the coherent radio emission could arise from the relativistic motion of bunches of particles along curved orbits; these orbits possibly may be identified with the field lines which emanate from the magnetic poles of a rotating neutron star and are able to cross the speed of light cylinder (open field lines). The incoherent optical and X-ray emission from NP 0532 can be produced by a simultaneous gyration of the particles around the same open field lines. Also, the radiation process should take place close to the speed of light distance $r = c/\Omega$ (Ω is the pulsar angular frequency) if it is caused by electrons. The pulses can, however, be emitted at a few stellar radii if the radiating particles are protons.

Particular pulsar models in which bunches of particles radiate in the radio band by virtue of the curvature of their orbits have already been discussed. Following a suggestion by Gold[1], Bertotti *et al.*[2,3] have considered the possibility that the pulses arise close to the speed of light distance, where the orbits should have a radius of curvature $\rho \sim c/\Omega$. As an alternative, Radhakrishnan[4] and Komesaroff[5] have suggested that the radiation is produced close to the star by particles moving away from the magnetic poles along the curved field lines. As the curvature of the lines of force at a distance r from the star is

$$\rho \sim \left(r\frac{c}{\Omega}\right)^{1/2}$$

these two alternatives are based upon the same idea; the difference is whether the curvature is evaluated close to the star or close to the speed of light distance.

We shall first show that the curvature of the field lines can be responsible only for the radio and not for the optical and X-ray emission from NP 0532. It is well known that the power radiated per unit frequency by a charge e moving relativistically

$$\left(\gamma = \frac{E}{mc^2} \gg 1\right)$$

along an orbit with radius of curvature ρ is given by

$$I(\nu) \sim \frac{1}{2\pi}\frac{e^2}{c}\left(\frac{\rho}{c}\right)\gamma\left(\frac{\nu}{\nu_{cr}}\right)^{1/3} \quad (\text{for } \nu < \nu_{cr}) \tag{1}$$

and reaches a maximum just below the critical frequency

$$\nu_{cr} \sim \frac{1}{2\pi}\frac{c}{\rho}\gamma^3 \tag{2}$$

The emission of frequencies up to about 10^{18} Hz at a rate of about 10^{36} erg s^{-1} would typically require $\gamma \sim 10^5$. Also the total number nV of radiating particles should be such that

$$\frac{1}{2\pi}\frac{e^2}{c}\left(\frac{c}{\rho}\right)\gamma nV \sim 10^{18}\ \text{erg s}^{-1}\ \text{Hz}^{-1} \tag{3}$$

Thus $nV \sim 10^{40} - 10^{41}$ particles (depending on where the emission occurs and the relevant value for ρ). For every conceivable value V of the emitting volume, this yields an implausibly high density of very energetic particles.

One is then led to consider the possibility that the optical and the X-ray emissions from NP 0532 are due to normal synchrotron radiation. Because the optical and radio pulses are simultaneous (at least within a small fraction of a millisecond), they should involve the same emitting region and, probably, the same particles. We shall consider a radiation scheme where the individual particles gyrate (with a small pitch angle) while also moving in bunches along the curved field lines. As we shall see, the observed brightness temperature in the radio band requires bunches much larger than the Larmor radius. One can therefore examine separately the two components of motion and the two radiation process. Also the emission connected with the gyration will be incoherent.

Pulses from the Speed of Light Distance

We examine first the case in which the pulses are emitted close to the speed of light distance and are connected with the kinematics described previously. When this possibility was considered earlier, Bertotti *et al.*[2,3] assumed for the magnetic field $B_\perp \sim B \sim 10^6$ G. This value close to the speed of light distance

$$r = \frac{c}{\Omega} = 1{\cdot}5 \times 10^8 \text{ cm}$$

corresponds to a surface strength around 3×10^{12} G (as deduced from the rate of slowing down). It is clear, however, that the optical and the radio pulses will coincide in timing and (roughly) in pulse width only if the radiating particles move with a small pitch angle θ around those field lines which we see during the radio pulse. If τ is the pulse width and P is the period, the requirement is $\theta \lesssim \tau/P$. In the case of NP 0532, the main pulse has a width $\tau \sim 250$ μs and period $P \sim 33$ ms; this implies $\theta \leq 5 \times 10^{-2}$ and $B_\perp \leq 5 \times 10^4$ G.

We note that, because of the additional contribution from the distribution of pitch angles, the optical pulse should still be wider than the radio pulse. This agrees with the observations.

Shklovskii[6] has recently used the theory of synchrotron reabsorption and the position of the low frequency cutoff in the optical spectrum of NP 0532 to determine $B_\perp$ in the emitting region. Assuming for this region a linear size $l \sim 10^7$ cm, he finds $B_\perp \sim 3 \times 10^3$ G which corresponds to a pitch angle $\theta \sim 3 \times 10^{-3}$. The observed optical and X-ray emission can then be due to electrons with an energy from ~ 100 MeV up to several BeV and a total density about 10^{14} cm^{-3}. Also, at the speed of light distance, the energy density in relativistic particles is almost as large as the electromagnetic energy density.

The value determined by Shklovskii is less than our upper limit and supports the idea of emission close to the speed of light distance. If indeed the pulses were emitted close to the star, the magnetic field would be stronger and the emitting region smaller, so synchrotron reabsorption would set in at frequencies much higher than observed. We stress, however, the assumption of an electron emission; as we shall see later, the position of the cutoff can be consistent with an emission close to the star if the emission is due to protons. For the moment, however, we shall accept the idea that the emission is due to electrons and occurs at a distance of order c/Ω.

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If the radio emission at frequency ν is attributed to bunches of particles moving along the field lines with velocity corresponding to a Lorentz factor γ_{bunch}, then the following condition should be satisfied

$$\frac{1}{2\pi}\frac{c}{\rho}\gamma^3_{\text{bunch}} \gtrsim \nu \tag{4}$$

The number of particles N contained in a bunch must be

$$N \gtrsim \frac{kT_{\text{b}}}{mc^2\gamma_{\text{bunch}}} \tag{5}$$

in order to achieve a sufficiently high brightness temperature T_{b}. The maximum dimension along the line of sight of a bunch which emits coherently at frequency ν is c/ν. In the case of a quasi-spherical bunch, this, together with relation (5), sets a lower limit to the particle density n.

If the individual particles have velocity v and pitch angle θ, then for the bunch

$$\gamma_{\text{bunch}} \leq \left(1 - \frac{v^2}{c^2}\cos^2\theta\right)^{-1/2} \tag{6}$$

which means roughly $\gamma_{\text{bunch}} \lesssim \min(\gamma_{\text{particles}}, \theta^{-1})$.

Coherent emission is observed up to about 10^9 Hz, corresponding to a wavelength of about 30 cm, so one requires $\gamma_{\text{bunch}} \gtrsim 300$. Some bunching should also exist with typical size $\lesssim 30$ cm. Because the brightness temperature of NP 0532 in the radio region is about 10^{26} K (again assuming an emitting region 10^7 cm), relation (5) gives $N \sim 10^{14}$. Note that there is no energy problem in the formation of these bunches—the kinetic energy of the particles exceeds the electrostatic energy. A volume $\sim$(wavelength)^3 and a density of 10^{14} cm^{-3} corresponds to a total number of particles per bunch in excess of 10^{18}. There would then be the required degree of coherence even if only 10^{-4} of the electrons present in the bunch had sufficiently small pitch angle to yield $\gamma_{\text{bunch}} \gtrsim 300$. It is also possible that the degree of coherence is reduced by the presence of positive charges in the bunch. (This would not affect the incoherent optical and X-ray emission.) The occasional presence of very strong radio pulses from NP 0532 (ref. 7) can be attributed to bunches containing a number of particles exceeding the typical value of 10^{14}. In the case of the Crab pulsar, this is energetically possible.

The spectrum of the pulsar radio emission should be determined chiefly by the degree of coherence at a given frequency, that is, by the spectrum of correlations in the plasma. In what follows we shall, however, use $N \sim 10^{14}$ as a typical coherence factor and we shall evaluate the density of the particles responsible for the radio emission by using equation (1). If we assume $\gamma_{\text{bunch}} \sim 300$, $N \sim 10^{14}$, $c/\rho \sim \Omega \sim 200$ s^{-1}, an emitting volume $V \sim l^3 \sim 10^{21}$ cm^3, and a luminosity 10^{31} erg s^{-1} over the radio band $\sim 10^9$ Hz, we have

$$\frac{1}{2\pi}\frac{e^2}{c}\left(\frac{c}{\rho}\right)^2 \gamma^4 n V N \sim 10^{31} \text{ erg s}^{-1} \tag{7}$$

This yields $n \sim 10^{12} - 10^{13}$ cm^{-3}.

We note that the formal rest plasma frequency exceeds the frequency of the radio waves. Because of the very strong magnetic field, high energies, and important collective effects, it is, however, clear that the usual simple theory of reabsorption cannot be applied here.

The pulsed nature of the source should be a consequence of the fact that the particles involved in the emission occupy a limited sector of the magnetosphere. Unfortunately, present knowledge of pulsar electrodynamics and the configuration of the field close to the speed of light distance does not allow any prediction to be made regarding the pulse structure and the duty cycle.

In the case of NP 0532, the main narrow pulse is preceded at radio frequencies by a much wider precursor. The observations available at present[7] show that the precursor is ~ 100 per cent linearly polarized while the main pulse seems to be much less polarized; and the precursor cannot be seen at optical and X-ray frequencies. We suggest tentatively that the precursor is due to protons streaming outwards along field lines different from those followed by the electrons[8]. If the Lorentz factor of the bunches containing an excess of protons also satisfies relation (4), then there would be proton radiation at the radio frequencies as well as the electron radiation previously considered; this is because the emission at radio frequencies depends on the curvature of the field lines and the Lorentz factor but not on the mass of the particles.

The different degree of polarization of the main pulse and the precursor can also be explained. It is well known that the radiation from charges moving along curved orbits is strongly polarized only close to or above the critical frequency. The observed difference in polarization between the main pulse and the precursor (if confirmed) could simply mean that, for the bunches, $\gamma_{\text{protons}} < \gamma_{\text{electrons}}$. In this case the proton radiation in the observed band could be closer to the critical frequency than the electron radiation and therefore more polarized.

If the acceleration in the near magnetosphere is chiefly electrostatic, one expects that the individual protons and electrons will reach about the same energy, that is, for the individual particles, $\gamma_{\text{protons}} \sim 10^{-3}\gamma_{\text{electrons}}$. In this case one should not expect any proton radiation in the optical or X-ray band. This might explain the absence of the precursor at high frequencies. The possibility of proton cyclotron or synchrotron radiation at very high radio frequencies or in the infrared is not worth consideration at present as the expected power output is very small.

Concerning the presence of an interpulse from NP 0532 (and from a few other pulsars) we simply remark that this could be due to a difference in the light travel time between two pulses emitted simultaneously from the two magnetic poles. (This would imply that the motion of the particles at $r \sim c/\Omega$ is such that we can see emission from both sides of the star.)

Finally, it is clear why only very fast pulsars are likely to emit optical or X-ray radiation. In the case of long period pulsars, the speed of light distance is much farther from the star and the local magnetic field strength at $r \sim c/\Omega$ is small so gyration therefore becomes less important. An emission spectrum with two peaks (one at radio frequencies due to the motion along the field lines, and the other at higher frequencies due to the gyration) would be a likely feature of most sources. One would expect the high frequency peak (proportional to B and therefore to P^{-3}) gradually to shift toward the infrared so that power emitted (proportional to B^2 and therefore to P^{-6}) gradually becomes negligible.

Pulses from Close to the Star

Komesaroff[5], following a suggestion by Radhakrishnan[4] has explored the alternative possibility that the pulses are emitted close to the star by bunches of particles moving away from the magnetic poles along the open field lines. As in the previous model, the curvature of the field lines produces the radio frequencies and the simultaneous gyration produces the optical and X-ray frequencies. An attractive feature of this model is the natural explanation for the duty cycle of the pulsars, because close to the poles the open field lines occupy only a small angular sector. This model can also account for certain features of the pulse shape and for the behaviour of polarization during the pulse. (We stress the implicit hypothesis that the field structure can be described by a simple dipole even close to the star.)

As we have already mentioned, however, the position of the low frequency cutoff in the optical spectrum of NP 0532 is not compatible with the idea of synchrotron radiation by electrons in the vicinity of the star. We wish to point out that this situation changes if the emission is due to protons. In this case the particles should have higher energies in order to produce optical and X-ray emission. In turn this permits a higher brightness temperature to be attained before self-absorption sets in.

To shorten our discussion, we give one set of parameters for radiation close to the star which appears to satisfy all the observational constraints.

Suppose that the emitting cone extends out to about five stellar radii (5×10^6 cm). The magnetic strength at this distance would be $B \sim 2 \times 10^{10}$ G, the field lines which eventually cross the speed cylinder would occupy a cone of about 10° and the diameter of the radiating area would be about 10^6 cm. By considerations analogous to those made earlier, one expects $B_\perp \lesssim 10^9$ G. It is then easy to use the formulae for synchrotron radiation and reabsorption (applied to protons) to find that it is possible to match the observed position of the low frequency cutoff in the optical spectrum.

The protons which radiate at optical and X-ray frequencies would have $\gamma \sim 30$ up to about 10^3 (energies up to about 1 erg). Assuming an emitting volume $(10^6)^2 \times (5 \times 10^6) \approx 5 \times 10^{18}$ cm^3, an output $\sim 10^{36}$ erg s^{-1} requires

$$\frac{e^2}{c}\left(\frac{eB_\perp}{m_p c}\right)^2 \gamma^2\, n\, V \sim 10^{36}\ \text{erg s}^{-1} \qquad (8)$$

that is, $n \sim 10^{17}$ cm^{-3}. For the anisotropic geometry of emission considered in this model, the total luminosity might be down by a factor of ten, that is, $L \sim 10^{35}$ erg s^{-1} and $n \sim 10^{16}$ cm^{-3}. This would correspond to an outward flux of energy in the form of relativistic particles $\sim 10^{38}$ erg s^{-1}, about the same as the overall energy requirements of the Crab Nebula. One must assume, however, that most of the energy is transferred to particles within only a few stellar radii.

Concerning the coherent radio emission, the radius of curvature of the relevant field lines at a distance $r \sim 5 \times 10^6$ cm from the star is 2×10^7 cm and therefore (from relation (4)) one should require $\gamma_{\text{bunch}} \gtrsim 200$. Because the brightness temperature of NP 0532 with an emitting region $(10^6)^2$ cm^2 would reach 10^{28} K, considerations similar to those made before require $\sim 10^{13}$ protons per bunch. The density of particles with pitch angle small enough to give $\gamma_{\text{bunch}} \gtrsim 200$ should be $\sim 10^{13}-10^{14}$ cm^{-3}.

We note that, if electrons are present, they would not affect the discussion of self-absorption because of the very high gyrofrequency. No simple solution can be offered, however, to explain the undetectability of electron radiation, unless the electrons move only along field lines which we are not able to see. Similarly, there is no simple explanation for the presence of a precursor in the radio pulse and its absence in the optical and X-ray band. One could instead explain the presence and the position of the interpulse if the geometry of the emission was such that the second pole also points (at some phase of each revolution) in a direction close to the observer. It is very likely in this case that one of the two poles will usually be better directed toward us than the other, so as to give rise to the main pulse. The emission from the pole which is less well directed would have to arise farther away from the stellar surface in order to be visible to us. Because of the smaller light travel time, the precursor would be expected to arrive sooner than halfway between two successive main pulses, and this is indeed the case for NP 0532.

Finally, we note that at the energies involved, nuclei would be completely stripped of surrounding electrons. This implies that similar considerations also apply to radiation from heavier nuclei, the maximum brightness temperature for incoherent synchrotron emission scaling as $m^{3/2}\,Z^{-1/2}$.

Pulsar Electrodynamics

As we have seen, the main advantage of explaining pulsars by radiation from close to the star is the simple interpretation of the duty cycle. One must assume, however, that we are seeing only radiation from protons (or heavy nuclei). If the radiation process occurs close to the speed of light distance, one can account for many features of the observations, but our present knowledge of pulsar electrodynamics is insufficient to explain the small duty cycle. In both cases, however, the pulsar radiation can be understood only if in the near magnetosphere (at distances $r \lesssim c/\Omega$) there are particles with Lorentz factors of about 10^3 and densities 10^{14}–10^{16} cm^{-3}. Some particles of higher energy should also be present. These high densities and energies would have important implications for the more general theory of pulsar electrodynamics.

We have already noted that, if the radiation is due to protons (within a few stellar radii) the energy loss associated with the outwards motion of these particles is about 10^{38} erg s^{-1}. The production of high energy particles (protons and, presumably, electrons) would then be chiefly a local phenomenon close to the star. The slowing down of the stellar rotation would also be related more to this ejection of particles than to the electromagnetic torques discussed in the past[8-10]. Well before the critical distance c/Ω, the particle energy density would indeed exceed the energy density in the electromagnetic field.

On the other hand, if the pulses are emitted at $r \sim c/\Omega$, the energy densities of the particles and the fields would be almost of the same order. If, at $r > c/\Omega$, the field decreases as $1/r$ (refs. 8 and 9) and the average energy per particle does not change appreciably, one could maintain this equipartition over the entire Crab Nebula.

At $r \sim c/\Omega$ one should expect also a deformation of the field lines with respect to the pure dipole case. We recall that, in a pure dipole field, the slowing down can be described by

$$\dot{\Omega} \propto \Omega^n \qquad (9)$$

where $n = 3$. The observed value $n \sim 2{\cdot}2$ (ref. 7) supports the notion of a distortion in the radial direction. (If fewer particles are ejected from old pulsars, one might expect that for them the index n will be closer to three.)

Finally, it is well known that the Crab Nebula contains some electrons with an energy up to about 10^{14} eV. Ostriker and Gunn[10] have shown how energies of this order can be reached when the particles move in the low frequency radiation field generated by the rotation of a neutron star with an inclined dipole field[11]. The basis of this acceleration is the existence at $r > c/\Omega$ of an electric field $E = B$ over a distance $\sim c/\Omega$. If the particles move in phase with the field, they would reach energies $E \times c/\Omega$ ($\sim 10^{14}$ eV in the Crab). The very high densities inferred from the pulsar radiation suggest, however, that only a few particles will be able to attain the maximum energy. The energy spectrum of the electrons in the Crab Nebula is therefore likely to arise from a combination of accelerations in the near and in the far magnetosphere.

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LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Hyperon Star Matter

HYPERON stars are neutron stars with a superdense core ($\rho > 10^{15}$ g cm^{-3}) consisting chiefly of baryons and heavier resonances. The equation of state for such a multicomponent system is determined by the elementary particle spectrum. This spectrum is known to be exponential up to about 2 GeV (ref. 1) For higher energies there is a worsening of experimental knowledge. We assume the exponential behaviour to continue (ref. 1 and unpublished work of Krzywicki) beyond 2 GeV, and take into account the fermions only, which yields in the low temperature limit the Hansen results[2]. Then the equation of state becomes temperature dependent because the heaviest components are partially nondegenerate. If the core of the hyperon star is in general relativistic thermal equilibrium, the polytropic index of the relation between pressure and total density (including kinetic energy) proves to be negative. As a consequence the velocity of sound in the radial direction, defined by

$$v_s = \sqrt{\frac{dP}{d\rho}},$$

decreases towards the centre instead of exceeding the velocity of light[3]. Under the additional hypothetical supposition that the mass spectrum remains exponential up to infinity (infinite energies), in hyperon stars the temperature cannot exceed a limiting temperature of $T_0 = 2 \times 10^{12}$ K and the time component g_{00} of the metric tensor of space-time cannot become zero.

For any gas component the conditions of thermal equilibrium in general relativity as given by Balasz[4], Ebert[5] and Ehlers (ref. 6 and a private communication) are

$$\gamma = \frac{\mu + mc^2}{kT} = \text{const}; \qquad T \cdot \sqrt{g_{00}} = \text{const} \qquad (1)$$

throughout the star, μ being the chemical potential, and m the mass of a hyperon of a single gas component. To obtain the equation of state we need the solutions of the relativistic Fermi-integrals, which were established by the quick numerical programme of W. D. Bauer (unpublished).

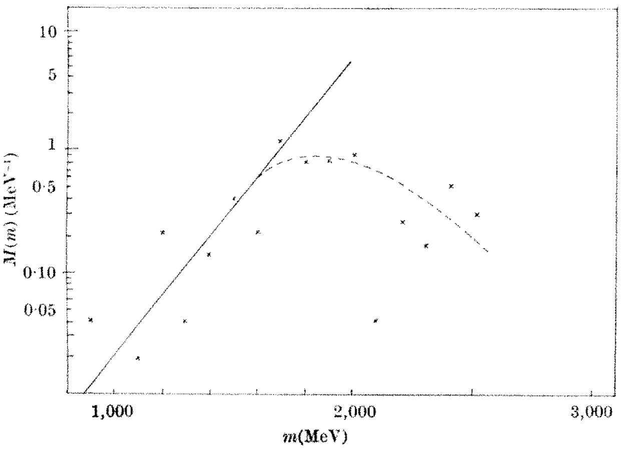

Fig. 1. The fit of the approximation of smooth abundance distribution to the experimental data.

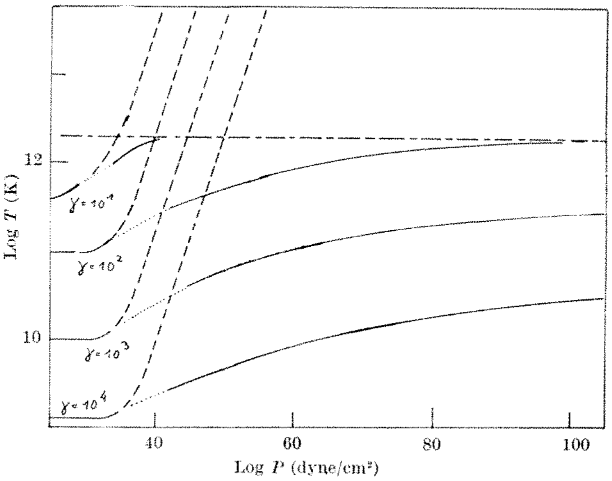

Fig. 2. The internal structure of hyperon stars.

If we neglect the virial interaction between the particles of a multicomponent system but take account of the baryon number and energy conservation, we can apply the local equilibrium condition

$$\gamma = \text{const}, \quad T = \text{const} \qquad (2)$$

for every single component. Eventually one can take into account the strong interaction between neighbouring particles by considering them as one new particle ("molecule"). For the level distribution of the components (of the multicomponent gas) Hansen's calculation proved that for high densities the antiparticles as well as the bosons diminish relative to the increase of fermions, which led us to our restriction to baryonic fermions.

The approximation of the smooth abundance distribution (the number of different hyperons per mass interval):

$$H(m) = \exp\left(\frac{mc^2}{kT_0} + 52\right), \quad T_0 = 2 \times 10^{12}\,\text{K} \qquad (3)$$

fits the experimental data well up to 2 GeV (Fig. 1). For higher energies the particle spectrum is poorly known. The parameter T_0 is almost identical with the "highest possible temperature in nature" as proposed by Hagedorn[1]. Using equations (3) and the relativistic Fermi-integrals and integrating over the whole hyperon spectrum, we obtain the equation of state of the multicomponent gas to be found in the interior of hyperon stars.

The internal structure of hyperon and (for comparison) of neutron stars is implicitly given by Figs. 2 and 3, because (because of conditions (1)) each γ-line has as a parameter the radius coordinate of a single star (if the general relativistic differential equations, connecting ρ, T, P, r, $M(r)$ have been solved).

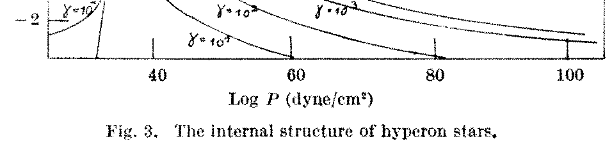

Fig. 3. The internal structure of hyperon stars.

From Fig. 2 it can be seen that in the centre of hyperon stars the temperature cannot exceed T_0 in contrast to the conventional neutron stars.

For $T \to T_0$ the matter curdles, the total rest mass energy grows faster than the kinetic energy. It can be seen from Fig. 3 that the exponent of the relation between P and ρ is less than 1 for every star, which indicates a negative polytrope index. In contrast to a pure neutron star, the log (P/ρ)-lines are dependent on γ which is due to the fact that the heaviest hyperon components are not degenerate.

In our multicomponent stars the velocity of sound in the radial direction $v_s = \sqrt{\frac{dP}{d\rho}}$ decreases with increasing density (towards the centre of the star) instead of surpassing the velocity of light as some authors[3] have proposed. Even if the density has a singularity at the centre, the time component g of the metric tensor remains nonzero.

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Astrophysical Implications of the Continuity Equation Plasma Oscillation

THERE are several periodic astrophysical phenomena which may involve the periodic generation of pulses of charged particles[1-5]. The periodicity of pulsars, for example, is usually attributed to a dynamical origin[6], but may instead have a plasma-physical origin. A characteristic feature of the continuity equation oscillation discovered recently is the periodic production of pulses of ions and electrons from the oscillating region. This oscillation arises from the time delay associated with the transit of charged and neutral particles across the oscillating region, which brings about a relaxation oscillation of the charged particle number density within it[7-9]

An appropriate physical model incorporating transit time delays has been analysed, and yields the continuity equations which follow (ref. 7 and my unpublished work).

$$\dot{n} = \hat{n}_e n(\sigma v)_{ne} - n_e n(\sigma v)_{ne} \qquad (1)$$

$$\dot{n}_e = -\hat{n}\, n_e(\sigma v)_{ne} + n n_e(\sigma v)_{ne} \qquad (2)$$

where $\hat{n}_e$ and $\hat{n}$ are, respectively, the time averaged electron and neutral number density in the oscillating region. $(\sigma v)_{ne}$ is the ionization rate parameter for electron-neutral ionization. It has been shown that the electron and neutral number densities oscillate with a frequency that is approximately

$$\nu = \frac{\sqrt{\hat{n}_e \hat{n}}\ (\sigma v)_{ne}}{2\pi}\ \text{Hz} \qquad (3)$$

when the peak-to-peak amplitude of $n_e(t)$ is small compared with its maximum value.

In an astrophysical context, the second terms of the right-hand side of equations (1) and (2) represent, respectively, the instantaneous rate at which neutrals are lost, and charged particles are produced, by electron-neutral impact ionization. My unpublished work shows that a consideration of the divergence term will yield the first terms appearing on the right-hand side of equations (1) and (2), when time delay effects are important. Equations (1) and (2) have long been known to possess periodic solutions[10-12]. The waveform of $n_e(t)$ is shown in Fig. 1 for a percentage ionization $\delta = 0{\cdot}01$, and for four values of the amplitude index $\eta = \hat{n}_e/n_{eo}$. The characteristics of the waveforms of $y(t)$ for $\eta \leq 0{\cdot}25$ are particularly suggestive of pulsar waveforms.

Comparison of the theory based on equations (1) and (2) with experimental observations has been reported elsewhere[8,9]. Data were taken which confirmed the functional dependence of frequency on the square root of the product $\hat{n}_e \hat{n}$ given by equation (3) (refs. 8 and 9). The frequencies observed in these experiments varied from 87 Hz to 100 kHz, a range of more than 10^6 in the density product $\hat{n}_e \hat{n}$. The waveform of the ion current flowing from the discharge was measured by a suitable collector. A representative sample of the data showing ion pulses from the discharge is given in Fig. 2 for the various background pressures of deuterium gas shown in Table 1. In each photograph, the time increases from right to left, and the zero level is the baseline between pulses. The pulses usually became broader and less regular as the

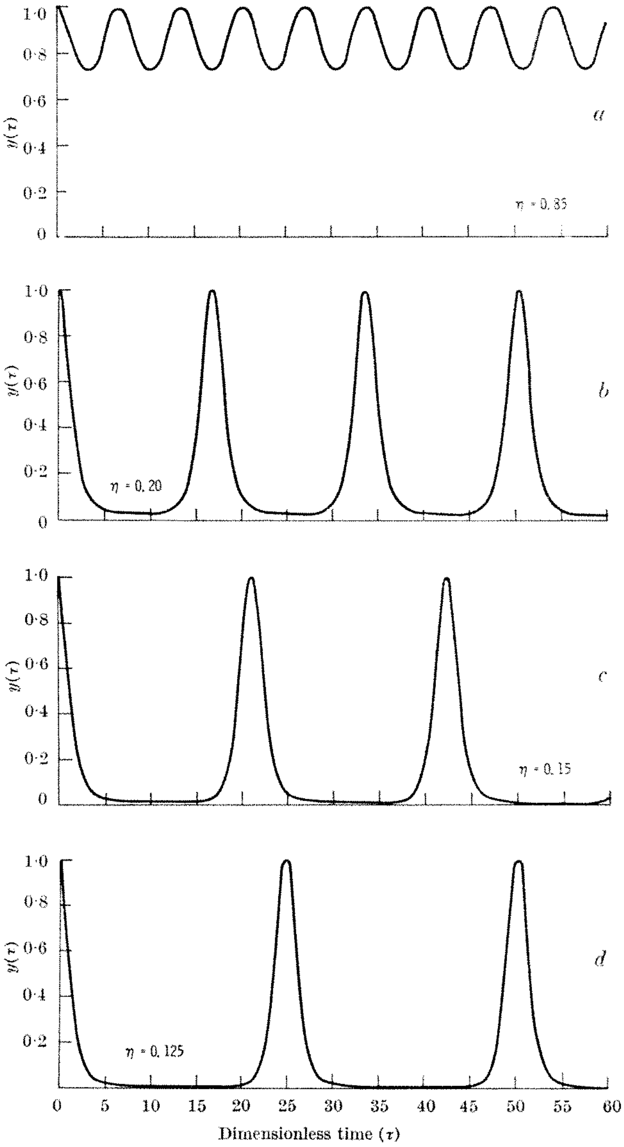

Fig. 1. Waveforms of the dimensionless electron number density $y(\tau)$, as a function of the dimensionless time τ, for selected values of the amplitude index, η, and for $\delta = 0{\cdot}01$. Note the sharp peaks separated by broad, shallow minima for low values of η.

Table 1. OPERATING CONDITIONS FOR WAVEFORMS OF $n_e(t)$ IN FIG. 3

Fig. No.	Indicated tank pressure (torr)	d.c. anode voltage (kV)	Average anode current (A)	y-axis scale (mV per major division)	z-axis scale (μs per major division)
2a	$2{\cdot}45 \times 10^{-6}$	15·0	0·0045	1·0	100
2b	$4{\cdot}75 \times 10^{-6}$	10·0	0·0040	1·0	50
2c	$4{\cdot}75 \times 10^{-6}$	15·0	0·0060	1·0	50
2d	$8{\cdot}0 \times 10^{-6}$	10·0	0·0055	1·0	20
2e	$1{\cdot}2 \times 10^{-5}$	10·0	0·0080	1·0	20
2f	$2{\cdot}2 \times 10^{-5}$	10·0	0·0140	1·0	20
2g	$2{\cdot}2 \times 10^{-5}$	25·0	0·0185	1·0	10
2h	$2{\cdot}65 \times 10^{-5}$	25·0	0·0390	5·0	10

neutral density was increased. The ion efflux in Fig. 2 shows such features as periodic interpulses (Fig. 2*b*), periodic fine structure on the individual peaks (Fig. 2*f*), and other details suggestive of pulsar waveforms. The sharp, narrow peaks between broad, flat minima of these waveforms are in good qualitative agreement with those predicted theoretically in Fig. 1.

The oscillation frequency was measured as a function of time, and is shown in Fig. 3. The frequency did not vary by more than a few per cent over a duration of 200 min, consistent with the precision with which n_e and n could be controlled in the experimental apparatus. This stability of the 28 kHz pulses for 200 min implies that the frequency did not change more than a few per cent over about $3{\cdot}3 \times 10^8$ individual pulses. This would imply a similar degree of stability for about 10 yr in an astronomical object with a period of one second.

Observations were made with a pair of collimated photomultipliers placed at various axial and radial positions. It was found that the plasma was oscillating in phase, with a single frequency. The single frequency was somewhat surprising in view of the approximately ten-fold variation in electron number density (and hence in the product $\bar{n}_e\bar{n}$) from the centre to the edge of the plasma. Apparently the fast-moving electrons serve as an averaging and synchronizing mechanism, and permit a region with large variations in $\bar{n}_e\bar{n}$ to oscillate as a macroscopic structure with a single eigenfrequency.

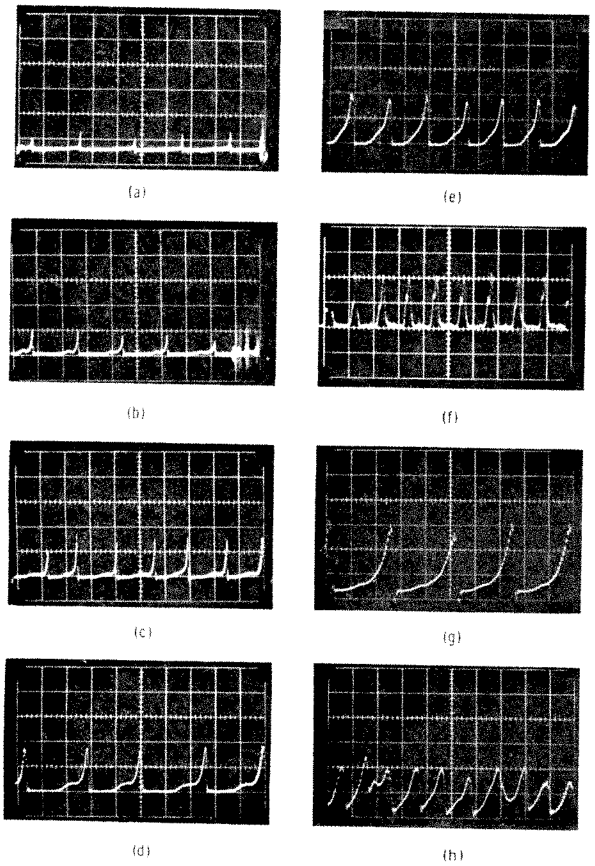

Fig. 2. Experimentally measured waveforms of pulsed charged particle flux from a discharge subject to the continuity equation oscillation. The experimental conditions for each case are listed in Table 1. All runs shown were with deuterium gas, at a maximum magnetic field of $B_{\max} =$ 1·0 Tesla. The charged particle number density is proportional to anode current. The zero level is the baseline, or lowest excursion, of the trace between pulses. Time increases from right to left.

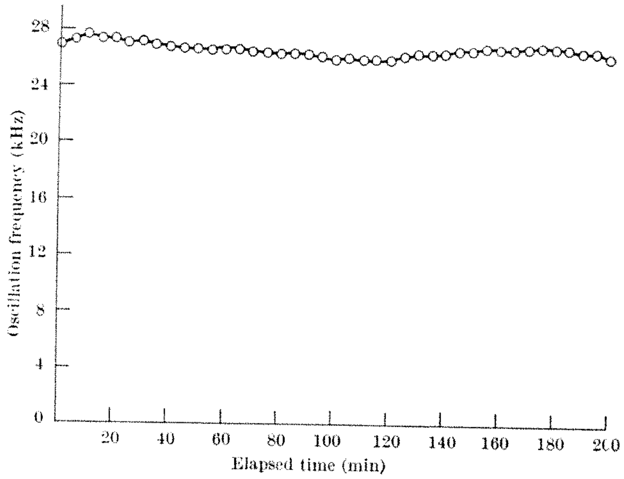

Fig. 3. Observed oscillation frequency for a 200 min experimental run.

It is a highly significant fact that, if the continuity equation oscillation is responsible for periodic astrophysical phenomena, a triggering signal is not required for the production of synchronized pulses, and the size of the pulsating object is not limited to the product of the fastest observed rise time and the speed of light. If the ionization rate parameters $(\sigma v)_{\rm ne}$ are the same, two spatially separated regions of partially ionized gas may behave like two independent clocks whose frequency of oscillation is determined only by the product $\bar{n}_e\bar{n}$. Electrons moving from one part of the region to another would be expected to bring the whole into phase, after an initial period of adjustment.

If a star possesses a strong magnetic field, it would undoubtedly also possess an extensive magnetosphere of trapped particles. The continuity equation oscillation may take place in the region of approximately constant $\bar{n}_e\bar{n}$ at the base of the magnetosphere, where the exponentially decreasing (with radius) neutral atmospheric density meets the increasing density of charged particles. Pulses of charged particles produced in such a region may well be heated by turbulent processes to the point where they emit pulses of r.f. and/or visible synchrotron radiation[13] in the stellar magnetic field.

Equation (3) implies that if the plasma were produced in some past catastrophic event, such as a supernova explosion, the period should lengthen as the electron number density decreases with time. This is consistent with the tendency for pulsar periods to lengthen[14,15]. It would not be too surprising to observe periods that shortened and then lengthened again as a flare or other event dumped an increased load of electrons into the stellar magnetosphere.

A similar mechanism may occur in the outer regions of a planet with a magnetosphere, at a radius where the particle mean free paths are comparable with the thickness of the oscillating layer. The periodic fluctuations of the charged particle number density would give rise to a periodic loading of the magnetospheric magnetic field lines, which in turn would cause geomagnetic micropulsations over a large region of the planetary surface. I have discussed further the application of the continuity equation oscillation mechanism to geomagnetic micropulsations and quasi-periodic scintillations of the interstellar medium in some unpublished work.

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Precession in a Disk Dynamo Model of the Earth's Dipole Field

THE Earth's dipole field has undergone more polarity reversals than was hitherto supposed, according to recent studies of geomagnetic data[1,2]. It has been estimated[2] that the Laschamp polarity interval lasted only about 20,000 yr, and Cox[3] has predicted that several intervals shorter than 50,000 yr remain to be discovered. I suggest here that such polarity intervals may be connected with the effects of precession acting on the Earth's dynamo process.

The question of whether precessional torques can affect the motion of the core has been widely discussed[4-7], following the work of Poincaré[8]. I am assuming that precession does affect the dynamo process in the core, and I show that this assumption may not be inconsistent with the recent data on short polarity intervals.

In his investigation of the lengths of polarity intervals, Cox[3] used the system of two coupled disk dynamos, which is known to exhibit magnetic field reversals[9-11]. One difficulty faced by this model is that the period of oscillation of the field is at most 50 yr, far too short to account for polarity intervals of the order of 10^4 yr. Bullard[4] suggested that the introduction of a Coriolis force might increase the periods considerably, but it is difficult to see how the effect of this force could be analysed without complicating the model considerably.

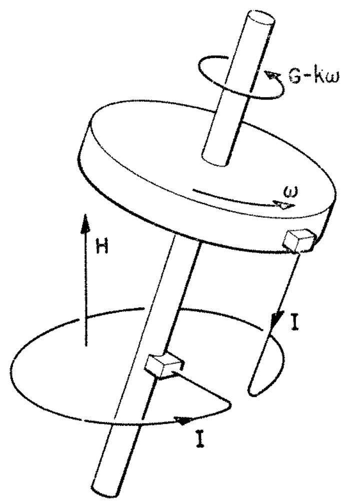

Fig. 1. Diagram of the precessing disk dynamo. The system is self-exciting if the directions of current I, magnetic field H and spin ω are as shown.

In the work described here, the effects of allowing a single disk dynamo to precess are investigated. This model does not exhibit field reversal but does possess an instability similar to that which immediately precedes field reversal in the coupled disk dynamo model. The precessing disk dynamo is shown in Fig. 1. The coil of wire remains fixed while the axis of the disk precesses about the normal to the plane of the coil. The ends of the coil brush against the axle and rim of the disk, utilizing the potential difference induced between these parts by the rotation of the disk in a small initial magnetic field H perpendicular to the plane of the coil. The resulting current I in the coil produces a field which tends to increase H. Given an initial field the system is therefore self-exciting, the energy being provided by a constant couple G applied to the axle of the disk.

The equations describing the model are[12]

$$L\dot{I} = \omega I M \cos\theta - RI$$

$$C\dot{\omega} = G - I^2 M \cos\theta - k\omega$$

$$A\ (\ddot{\theta} - \dot{\phi}^2 \sin\theta \cos\theta) + C\omega\dot{\phi} \sin\theta = mgl \sin\theta$$

$$A\ (\ddot{\phi} \sin\theta + 2\dot{\theta}\ \dot{\phi} \cos\theta) - C\omega\dot{\theta} = 0$$

where the equations of motion have been written using axes that precess with the body; ϕ measures the angular precession and θ the angle between axle and normal to the plane of the coil. The axle is pivoted about a point distance l below the centre of mass of the axle and disk and (A, A, C) are the moments of inertia of the body (of mass m) about the precessional axes. A viscous force $k\omega$ opposes the rotation of the disk. The current circuit has resistance R and self-inductance L, and the mutual inductance between the coil and the rim of the disk is approximated by $2\pi M \cos\theta$ (M remains constant).

The equations may be written in non-dimensional form by introducing four dimensionless parameters. The quantity most difficult to estimate is the ratio L/M. Values assigned to L/M in previous work vary between 1 (Rikitake[11]) and 100 (Allan[9]). In this range of L/M numerical integration of the equations shows that the periods τ_c of current oscillations are longer than corresponding ones in the ordinary disk dynamo by a factor of five at most.

The interesting feature of the model is, however, that τ_c is between 0·3 and 3·0 times the precessional period (τ_p) corresponding to values of L/M between 1 and 100. If one uses the value of τ_p for the Earth ($2{\cdot}6 \times 10^4$ yr) as a measure of τ_c, one obtains values of between $7{\cdot}8 \times 10^3$ yr and $7{\cdot}8 \times 10^4$ yr. From the small amount of data on recent oscillations of the dipole moment it seems that τ_c is about 10^4 yr. Thus if the appropriate value of L/M for the Earth's core is about 10, the ratio τ_c/τ_p for the model is in reasonable agreement with the available data. A first-order stability analysis[12] favours the lower values of L/M because the equilibrium value of the (dimensionless) angular velocity goes as $(L/M)^{-1/2}$. Thus the larger L/M is, the slower the disk spins. Instability therefore becomes more likely as the value of L/M is increased.

The suggestion regarding this instability is supported by the numerical integrations[12]. Even with $L/M = 1$ an initial disturbance in the angular velocity amounting to 10 per cent of its equilibrium value is enough to cause an appreciable increase in the current after three oscillations. Although the current cannot reverse in this model, it is worth noting that in the two-disk model a true reversal (as defined by Allan[9]) is always preceded by an increase in the amplitude of the current oscillations. If the increase in current after three oscillations obtained for the case $L/M = 1$ is enough to cause reversal, an initial polarity interval of about 23,000 yr would be implied.

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Deposition of Manganese Rich Sediments during Glacial Periods

SEVERAL elements, such as manganese, vary considerably in concentration in different sediments. Factors such as chemical weathering, run off from the continents and physicochemical conditions in the transport medium or in the region of deposition may cause these variations, and volcanic and biological processes may also produce manganese-enriched deposits. The effect of such processes on the areal distribution of manganese has been extensively studied, but their effect on the temporal distribution of manganese is little known. Wangersky and Hutchinson[1] found that Caribbean sediments from the last four glaciations are rich in manganese. My data indicate that manganese, copper and calcium carbonate are enriched in sediments from most glacial periods in the Caribbean.

For this study, the Caribbean core *P*6304–9 (14° 57′ N and 68° 55′ W at a depth of 4,126 m) was chosen because eight glacial events are recorded in it[3]. The core was sampled every 20 cm and analysed for $CaCO_3$, NaCl, Mn, Fe, Cu, Y, La, Co and so on. Emiliani determined $^{18}O/^{16}O$ ratios[2] and carbonate concentration (unpublished). $CaCO_3$ was analysed in both cases by the acetic acid leaching technique[3]; the agreement between the two sets of analyses is good. Mn and Fe were determined by both atomic adsorption and emission spectroscopy, and Cu, Y, La and Co by emission spectroscopy. The analytical precision is 2–3 per cent for $CaCO_3$; 10 per cent for Cu, Mn, Co and Fe; and about 20 per cent for Y and La. Most analytical results are shown in Fig. 1, together with the isotope data from Emiliani[2]. Because of scarcity of the material, $^{18}O/^{16}O$ ratios, $CaCO_3$ content and other data had to be determined from different samples. Moving averages involving three consecutive values each time were used to eliminate short term noise in the data without destroying the cyclic patterns (see Fig. 1), because each cycle involves seven to fifteen data points. The trend in the $CaCO_3$ data was eliminated by means of the correction factor $y = +0{\cdot}0074\,x$ (y = content of $CaCO_3$ (in per cent), x = depth in the core (in cm)) which was derived from the trend equation for $CaCO_3$, $y = 57{\cdot}8 - 0{\cdot}0074\,x$. Correlation coefficients were calculated, using the data processed in this way (Table 1).

The curves for $\delta^{18}O$/PDB–1 ($\delta^{18}O$ parts per thousand, with respect to the Chicago Carbonate Standard), Mn, Cu and $CaCO_3$ show cyclic patterns that cofluctuate, particularly in the deeper sections of the core. All data for Mn, Fe, Cu, La, Y and Co discussed here refer to carbonate free basis. The correlation between manganese and $\delta^{18}O$/PDB–1 maxima at 400, 820, 975 and 1,300 cm (see Table 1) is most striking. Less pronounced correlations with $\delta^{18}O$/PDB–1 are shown by maxima in the $CaCO_3$ and

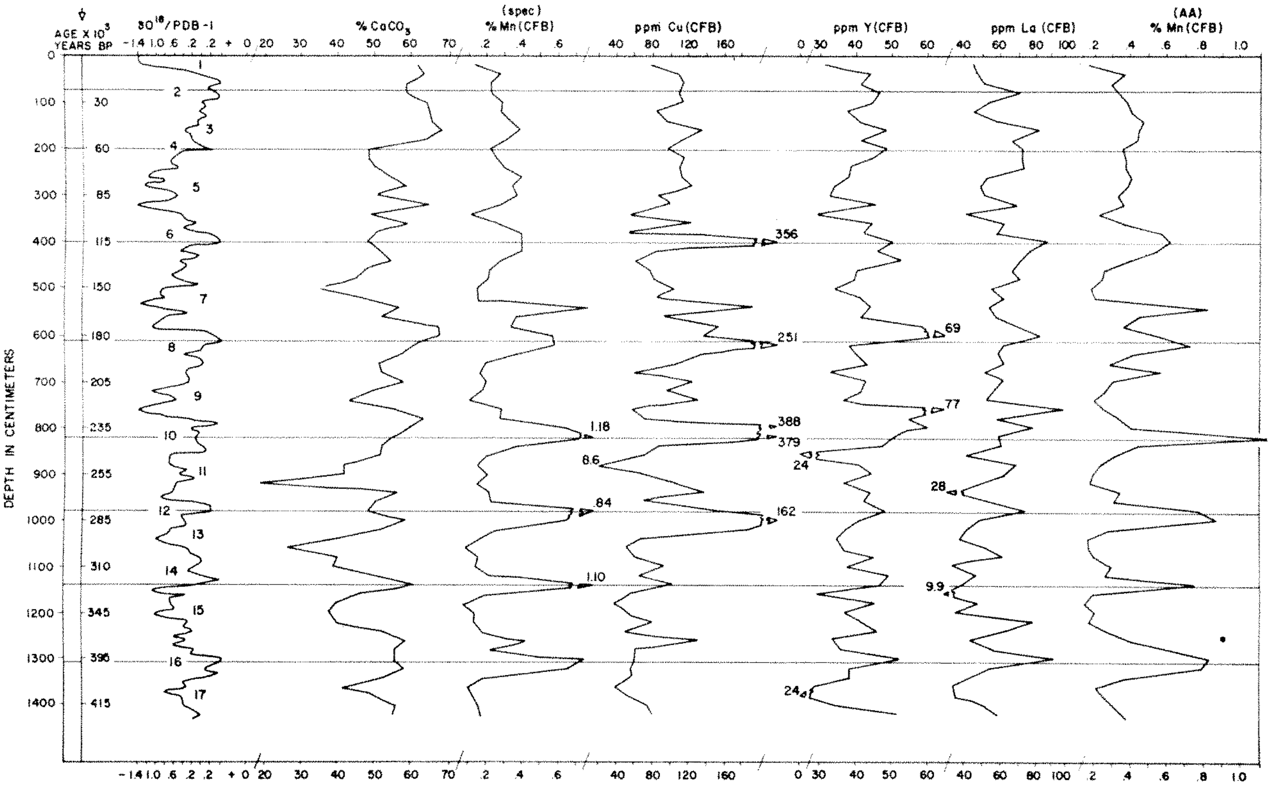

Fig. 1. Analytical results for core *P*6304–9; $\delta^{18}O$ and $CaCO_3$ from Emiliani[2]. Mn (per cent), Cu, Y and La (p.p.m.) are given on a carbonate free basis (CFB). Mn was determined by spectroscopic analysis (spec) and atomic absorption measurements (AA), showing a very good agreement. Two other series of manganese analyses and another series of copper analyses are not shown in the figure but duplicate the data in the Mn (spec) and Cu (spec) curves.

Table 1. CORRELATIONS BETWEEN $\delta^{18}O$/PDB–1, $CaCO_3$ AND METALLIC ELEMENTS IN CORE *P*6304–9

	1 (15)	2 (9)	3 (12)	4 (8)	5 (7)	6 (8)	7 (10)	8 (54)	9 (44)	10 (69)
$\delta^{18}O$/PDB–1–Mn	−0·45	0·88‡	0·21	0·80†	0·75†	0·58	0·73†	0·51‡	0·50‡	0·37‡
$\delta^{18}O$/PDB–1–$CaCO_3$	0·53†	0·26	0·52	0·39	0·51	0·13	0·42	0·32†	0·26	0·35‡
$\delta^{18}O$/PDB–1–Cu	—	0·81‡	0·55	0·76†	0·36	0·74†	—	—	0·26	—
$\delta^{18}O$/PDB–1–La	0·07	−0·03	0·35	−0·08	0·90‡	0·29	0·61	0·20	0·15	0·17
$\delta^{18}O$/PDB–1–Co	−0·08	−0·24	−0·02	0·81†	0·81†	−0·59	−0·16	0·05	0·12	0·04
Mn–$CaCO_3$	0·17	0·38	0·71‡	0·75†	0·76†	0·89‡	0·71†	0·63‡	0·69‡	0·58‡
Mn–Cu	—	0·88‡	0·82‡	0·98‡	0·77†	0·72†	—	—	0·59†	—
Mn–La	0·14	−0·06	0·50	0·31	0·88‡	−0·53	0·78‡	0·29†	0·23	0·27†
Mn–Co	−0·18	−0·05	0·88‡	0·97‡	0·87‡	−0·51	0·30	0·56‡	0·69‡	0·55‡
$CaCO_3$–Cu	—	0·13	0·73‡	0·67	0·74*	0·49	—	—	0·37†	—
$CaCO_3$–La	−0·30	−0·37	0·68†	0·60	0·53	−0·81†	0·39	0·28†	0·28	0·23
$CaCO_3$–Co	−0·64‡	0·32	0·69†	0·72†	0·69	−0·29	0·00	0·48‡	0·65‡	0·41‡
Cu–La	—	−0·06	0·58†	0·33	0·50	−0·33	—	—	0·06	—
Cu–Co	—	0·18	0·60†	0·95‡	0·63	−0·89‡	—	—	0·42‡	—
La–Co	0·18	−0·64	0·27	0·44	0·86*	0·29	0·48	0·30†	0·28	0·27†

* Close to but above the 5 per cent significance level. † Significant on the 5 per cent level. ‡ Significant on the 1 per cent level.

Column 1 gives correlations for the layer 40–320 cm, 2 for 340–500 cm, 3 for 520–740 cm, 4 for 760–900 cm, 5 for 920–1,040 cm, 6 for 1,060–1,200 cm, 7 for 1,220–1,400 cm, 8 for 340–1,400 cm, 9 for 340–1,200 cm and 10 for 40–1,400 cm. Figures within parentheses at tops of the columns indicate number of samples. Y shows the same correlations as La, probably because of the similarity in distribution patterns for Y and La.

Cu (Table 1). The correlation coefficient between $\delta^{18}O$/PDB–1 and Cu in column 9, Table 1, does not quite reach the 5 per cent significance level, possibly because no long-trend correction was made for Cu, but other calculations indicate that Cu is better correlated with $\delta^{18}O$ than is $CaCO_3$. Thus the absolute discrepancy in peak positions for Mn etc with respect to the $\delta^{18}O$ peaks can be expressed as a fraction $|\Delta\lambda|$ of the length λ of the cycle in which the $\delta^{18}O$ peak under consideration is located. (The error in the definition of λ for each cycle affects all peak discrepancies equally and is of little consequence.) The standard deviation of these peak discrepancies is 0·06 (for Mn–$\delta^{18}O$), 0·12 (for Cu–$\delta^{18}O$) and 0·18 (for $CaCO_3$–$\delta^{18}O$). For Y, La and Co the correlation with $\delta^{18}O$/PDB–1 is poor and Fe showed no correlation.

The oxygen isotope ratio has been used as a palaeothermometer[2], the positive peaks in the $\delta^{18}O$/PDB–1 curve corresponding to cold periods. These data and earlier reports[1,4] suggest that variations in the content of $CaCO_3$ (total sample) and Mn and Cu (carbonate free fraction) are partly climatically controlled. The Mn and Cu content seem to be better climate indicators than the $CaCO_3$ content.

The negative correlation in layer 40–320 cm for ($\delta^{18}O$/PDB–1)–Mn may be caused by some changes in deposition conditions or post-depositional migration or disturbance during sampling, because in core *A*172–6 the events 1, 3 and 5 (ref. 2) are better resolved than in *P*6304–9. Turekian reached similar conclusions for the Equatorial Atlantic Core 180–74 (ref. 5). In the layer 340–1,400 cm the correlation coefficient 0·51 compares favourably with previous results[1].

The correlations between $CaCO_3$–Mn and $CaCO_3$–Cu may not suggest any geochemical affinity between these constituents, but they may be caused by the same controlling climatic factors. The high correlations between the elements Mn–Cu, Mn–Co and Cu–Co are in agreement with what is known about their geochemistry[6].

The formation of manganese rich sediments during glacial periods could possibly be the result of an increase in chemical weathering during these periods caused by pluvial conditions which occurred at lower latitudes simultaneously with the glaciations at higher latitudes[7]. The increase in run off during the glaciations[5,7] could have coincided with an even more accelerated weathering rate. The connexion between pluvial and glacial events does not seem to be fully understood, however[7–10]. Another explanation could be that during interglacial periods, laterites and related weathering products are formed and are brought into the sea by increased run off from the continents during glacial periods. A third explanation could be a more complete removal of manganese from seawater during cold periods. Ocean water contains about 0·002 mg Mn/l. During a cold period of 20,000 years a water column of 3,800 m will deposit at the most 0·012 g Mn/cm², because the residence time of manganese is about 1,300 years[11]. The amount of manganese deposited during such a period is about 0·12 g Mn/cm², however, assuming that dry uncompressed sediment *in situ* contains 60 per cent $CaCO_3$ and has a density of about 0·75. Present weathering conditions are consequently unable to support the deposition rates during the pluvials unless manganese was preferentially deposited in this area.

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Finite Elements for Geological Modelling

SCALE models have often been used to illustrate or gain insight into geological processes[1,2]. It may now be possible to study full scale models using a digital computer and the finite element method—a method of stress analysis in complex structures developed in the engineering sciences[3].

Fig. 1 represents a cross-section through part of the Earth's crust: the thickening of the crust at *A* and *B* is intended to represent mountain chains with compensating roots on the Airy principle of isostasy[1]. The length of the cross-section is 800 km and the minimum thickness of the crust is 25 km (vertical exaggeration 4 : 1). With this simple model the finite element method could be used, say, to investigate the effects of deposition in the broad trough between the two mountain ranges, accompanied if required by erosion of the mountain ranges. The effects of tectonic forces could also be included. Here I simply illustrate the method by considering deposition in the trough.

The finite element method requires that a continuous structure be replaced by a series of elements (the tri-

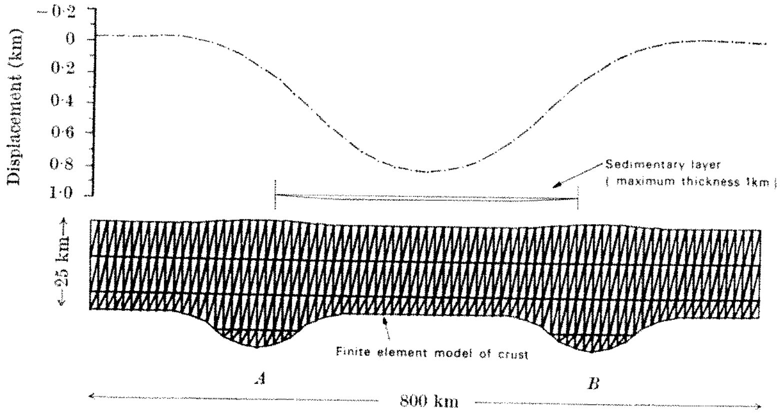

Fig. 1. Cross-section through a finite element model of the Earth's crust with computed displacement from loading with a lens of sediment.

angles of Fig. 1) which are assumed to interact at only a few points called nodes (the vertices of the triangles). Then, whereas the displacements in a continuum must be specified at an infinite number of points, displacements in the finite element method are specified in terms of the displacements of the nodes. For each element a stress–nodal displacement relationship is formulated in terms of the elastic constants and dimensions of the elements, and these relationships are assembled into a larger set of equations which describe the deformation of the whole structure under load. These equations have the form:

$$[\mathbf{F}] = [\mathbf{K}]\ [\mathbf{Y}]$$

where [**F**] is the load vector (including body forces), [**Y**] the displacement vector and [**K**] the stiffness matrix. To include the effects of buoyancy (the crust is supported by a viscous substratum on the classic theory of isostasy[1]) the vertical component of the load vector acting at the base of the crust is made to vary linearly with the vertical displacement.

To simplify the computation, the cross-section of the model (Fig. 1) is constant at right angles to the cross-section. Simple triangular elements can then be used, the displacements of the nodes being confined to the plane of the cross-section. This is the condition of plane strain.

Using a program given by Zienkiewicz[3] I have computed the displacements of the model (Fig. 1) resulting from the deposition of a lens of sediment (density 2·6 g/cm^3) 1 km thick at the centre of the trough and thinning towards the flanking ridges. (The load of sediment is actually replaced by equivalent vertical point forces acting at the appropriate nodes.) The program was modified to include the effects of buoyancy, the density of the crust was taken as 2·8 g/cm^3 and the density of the substratum as 3·3 g/cm^3. A Young's modulus of 10^{12} dynes/cm^2 and a Poisson ratio of 0·25 were taken for the elastic constants of the crust. The crust has been assumed to be uniform, but this is not essential, and each element could have different elastic properties: in this way geological structures can be simulated. Elements with zero strength can be used to simulate faults (Zienkiewicz[3]).

Fig. 1 shows the lens of sediment and the computed deformation of the crust resulting from the loading. As would be expected, the greatest deformation is at the centre of the model. Small uplifts of the crust on the flanks of the downwarp also occur. Similar effects have been found by Vening-Meinesz[4] in his classical studies of the deformation of a uniform rigid crust under loading. The maximum depression of the crust in the model is 0·81 km, about what one would expect: a uniform crust floating in a viscous substratum of density 3·3 g/cm^3 would subside by 0·79 km under loading with a uniform layer of sediment 1 km thick and density 2·6 g/cm^3. The crust as a whole proves to be in equilibrium under the sedimentary load because $\rho_s A_s = \rho_v A_v$ as it should (ρ_s is the density of the sediment, A_s the cross-sectional area of the sedimentary layer, ρ_v the density of the substratum and A_v the integral of the displacement of the base of the crust over the whole cross-section).

The model described here is crude, but its behaviour under load suggests that, with refinement, it could be used to study the build-up of stresses within the crust as a result of the relatively simple processes of erosion and deposition. This may show that, for example, some rift valleys are explicable as the results of differential loading of the crust. The effects of deposition along the continental margin could also be modelled. If these studies give useful results increasingly more complex models can be investigated: the effects of elastic anisotropy, heat flow, fluid flow through pores and nonlinear deformation are already being included by civil engineers in finite element models[3]. By incorporating more and more of the accumulated mass of geological, geophysical and geomorphological data, very elaborate models can probably be constructed with which to test tectonic hypotheses. This should help to show which of the many hypotheses are consistent both within themselves and with the observational data.

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Continental Climatic Variations between 130,000 and 90,000 Years BP

CLIMATIC variations in the Quaternary have so far been described with some precision, in the range of 0 to 35,000 yr BP, by radiocarbon dating and by using the isotopic ratio $^{18}O/^{16}O$ to estimate temperature. The ratio was measured either in foraminifera[1] for the marine environment, or in stalagmites[2-4] for the continental environment.

Corals, which may be dated back to about 500,000 yr BP by the uranium desequilibrium method, may be used to determine dates outside the range of ^{14}C measurements. These corals also reflect sea level variations associated with temperature. Variations of foraminiferal associations[5] or their isotopic composition[6] can also be used, but radiometric methods are not very accurate in these cases. More often, mean sedimentation rates have been assumed. From these data, correlations with the Milankovitch summer insolation curve, a cyclic variation of 21,000 yr related to the precession and a period of 41,000 yr due to the tilt of the Earth's axis[7], have been attempted[8].

Accurate data are therefore necessary both for dating and for temperature evaluation for periods outside the range of ^{14}C dating, and especially for the periods of transition between warm and cold periods.

We have previously shown that, in certain conditions, $^{18}O/^{16}O$ ratios in stalagmites give a good indication of the mean temperature of the air above the cave[9] and that old stalagmites may be dated by the $^{230}Th/^{234}U$ method[10].

In the Aven d'Orgnac (43° 50′ N, 4° 30′ E) in southern France, we found a stalagmite which was formed between

130,000 and 90,000 yr BP, during the interglacial Riss/Wurm. This stalagmite is 244 cm high, very pure, and has regular cylindrical shape about 10 cm in diameter. It was growing about 3 km from the entrance of the cave, and, consequently, out of the influence of the outer atmosphere. Samples for dating were taken from the central part of the column to avoid contamination by clay or by secondary crystallization in the outer edges. Isotopic analyses were performed on 30 mg samples taken from the axial part of the stalagmite, because, in this part, the isotopic composition of the calcite was highly reproducible[9]. Each sample was treated, at 25° C in vacuum, by 100 per cent H_3PO_4, and the CO_2 obtained was then purified and analysed with an Atlas *M* 86 mass spectrometer.

Table 1 gives the results of uranium dating and of $^{18}O/^{16}O$ measurements, and Fig. 1 gives the curve of variations of $\delta^{18}O$ with time.

We have previously shown[9] that the oxygen composition in a cave is related to temperature variations only if there is no air movement and no relationship between carbon fractionation and oxygen fractionation. Fig. 2 shows that there is no correlation ($r = 0{\cdot}33$) and in this case the variations of $^{18}O/^{16}O$ do represent temperature variations.

Table 1

Levels (cm from the top)	^{238}U (p.p.m.)	Activity ratio (actual) $^{234}U/^{238}U$	Activity ratio (actual) $^{230}Th/^{234}U$	Age* (10^3 yr)	$\delta\,^{18}O$ PDB
2·4					−4·38
4·8					−4·48
7·2					−5·74
12·8					−5·67
13·4	0·070	1·19 ± 0·03	0·58 ± 0·01	92 ± 2	
29·2					−5·45
30·4					−5·74
35·2					−5·95
44·0					−6·31
47·2					−6·13
53·6					−5·77
56·8					−5·69
60·0					−5·91
69·6					−5·90
72·9	0·070	1·04 ± 0·02	0·58 ± 0·02	93 ± 4	
73·2					−6·00
76·0					−5·72
80·0					−6·02
85·2					−5·81
87·2					−5·21
92·0					−5·03
92·8					−4·83
95·2					−4·60
98·2					−4·68
100·0					−4·53
104·0					−4·60
108·0					−4·77
111·4					−4·46
113·2					−4·55
122·0					−4·56
126·0					−4·47
128·0					−4·55
128·8	0·064	1·10 ± 0·03	0·60 ± 0·01	100 ± 2	
130·4					−4·48
134·0					−4·74
144·0					−4·56
148·0					−4·50
164·0					−4·52
168·2					−4·82
173·2					−4·63
182·0					−4·78
190·0					−4·59
193·5	0·066	1·05 ± 0·02	0·66 ± 0·02	120 ± 6	
196·0					−4·55
202·4					−5·28
209·2					−5·43
222·8					−5·34
230·0					−5·50
233·4	0·066	1·10 ± 0·03	0·70 ± 0·01	129 ± 5	
234·0					−5·94

* Using the relationship

$$\left[\frac{^{230}\mathrm{Th}}{^{234}\mathrm{U}}\right]_{\text{measured}} = \frac{(1 - e^{-\lambda_{230_{\mathrm{Th}}}t})}{(^{234}\mathrm{U}/^{238}\mathrm{U})_{\text{meas.}}} + \left[1 - \frac{1}{(^{234}\mathrm{U}/^{238}\mathrm{U})_{\text{meas.}}}\right]$$

$$\left[\frac{\lambda_{230_{\mathrm{Th}}}}{\lambda_{230_{\mathrm{Th}}} - \lambda_{234_{\mathrm{U}}}}\right]\left[1 - e^{-(\lambda_{230_{\mathrm{Th}}} - \lambda_{234_{\mathrm{U}}})t}\right]$$

$$T_{230_{\mathrm{Th}}} = 75{,}200 \text{ yr}$$

$$T_{234_{\mathrm{U}}} = 2{\cdot}48 \times 10^5 \text{ yr}$$

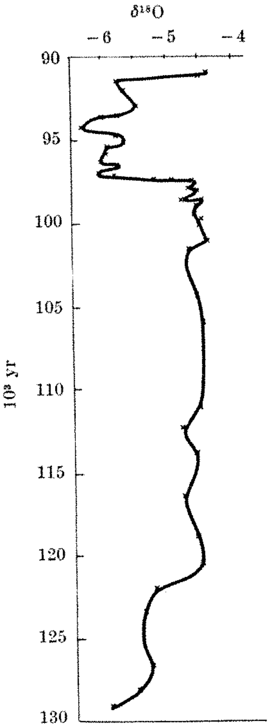

Fig. 1. Variations of $\delta^{18}O$ versus ages in the Orgnac stalagmite.

Dansgaard[11,12] has shown that Greenland ice has low ^{18}O concentrations when it corresponds to a cold period. Assuming that this phenomenon is general for rain waters in high and middle latitudes, the Dansgaard relationship gives an enrichment of 0·69 ‰ in ^{18}O for an increase of the temperature of 1° C. These results will be more precise when the isotopic and thermal variations of oceanic waters are better known, because the isotopic composition of rain water depends on these factors.

The fractionation of oxygen at the moment of crystallization of calcite is also an important factor. McCrea[13] has shown that in equilibrium conditions, an increase of 1° C in the temperature of the environment produces a 0·24 ‰ decrease in the fractionation factor in the calcite.

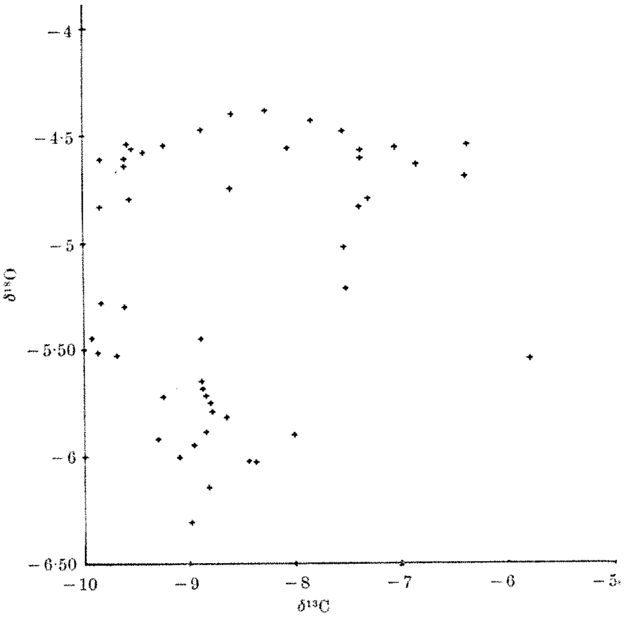

Fig. 2. Correlations between $\delta^{18}O$ and $\delta^{13}C$ in the Orgnac stalagmite.

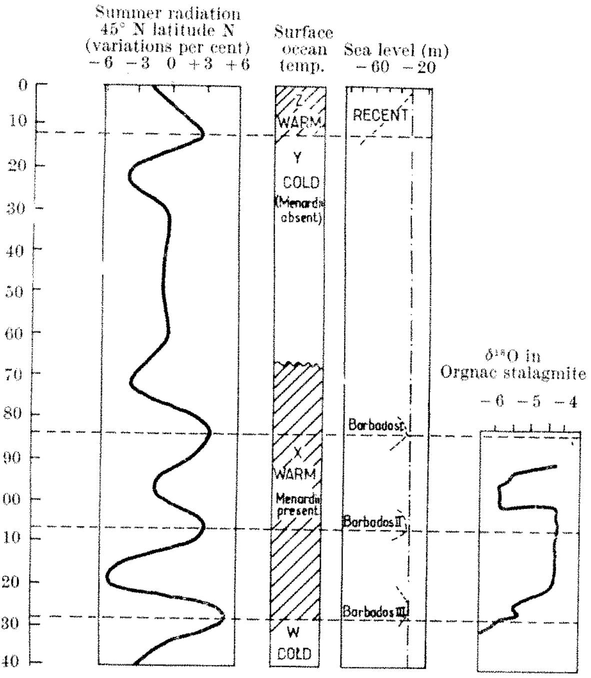

Fig. 3. Correlations between the variations of intensity of solar radiations, surface temperature of the oceans, the sea levels (from Broecker[8]) and the variations of $\delta^{18}O$ of the Orgnac stalagmite.

If the temperature above the cave varies by Δt, these hypotheses give the following relationship

$$\Delta^{18}O = 0{\cdot}69\,\Delta t - 0{\cdot}24\,\Delta t$$

That is

$$\Delta^{18}O = +0{\cdot}45\,\Delta t$$

On our curve, the maximum variation in temperature is 4° C. The temperature increased slowly (4° C in 10,000 yr) from 130,000 to 120,000 yr BP. It then remained approximately constant, with only minor variations ($<0{\cdot}5$° C) from 120,000 to 97,000 yr BP. This relatively warm period ended abruptly 97,000 yr ago when, in about 1,000 yr, the temperature fell about 2·5° and was followed by variations, the minimum being about 95,000 yr ago. Between 93 and 92,000 yr BP, that is in 10^3 yr, the temperature rose by about 4° C.

The first conclusion that may be drawn from this curve is that the time constants of climatic phenomenon may be very short. There is also no correlation between the Milankovitch curve and our curve, and it is evident that there is no 21,000 yr periodicity. Unfortunately, our record is too short to study the possibility of a 40,000 yr periodicity. This result agrees with the conclusion of Shaw and Donn[14] that the Milankovitch effect is too small to have induced glaciations.

If, according to Broecker's hypothesis, we assume that the Ericson X zone represents Wurm Riss interglacial, our curve shows the start of rewarming and covers a great part of the warm period. It is possible that the short cold episode, about 95,000 yr ago, was too short to have influenced the foraminiferal fauna of the Caribbean sea.

The high sea level represented by Barbados III may be correlated with the rapid increase of temperature between 125 and 120,000 yr BP, but it is not so easy to explain another high sea level corresponding to Barbados II. Minor variations of the sea level may have occurred, and this would explain, as Barbados II is lower than Barbados III, that Thurber *et al.*[15] and C. L. *et al.*[16] have found in atoll drills only one high sea level about 100,000 yr ago, which corresponds to the end of a warm period. Rona and Emiliani[17] have also found that a cold period occurred about 100,000 yr ago.

We thank the Municipality of Orgnac for allowing us to work in the cave and to sample stalagmites. We also thank M. Guy Rieu for his help and Mrs Paulette Martineau for helping us to prepare the samples.

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Variation in Weather-type Temperature Averages

Barry and Perry[1] describe an analysis which asserts that the mean temperatures of weather-types exhibit a "within-type" change between 1925–35 and 1957–67 at Eskdalemuir (55° 19′ N, 03° 12′ W, 242 m) for January, April, July and October. The authors concluded that Dzerdeevskii's hypothesis[2]—that all changes in temperature and precipitation are associated with changes in frequency and duration of large-scale circulation patterns—needs to be modified at least on the local scale in Britain. Some of the procedures used are, however, open to criticism and their conclusion may therefore be invalid.

Possible sources of error or difficulties in assessing the results might arise from changes of site, surroundings, observational procedure and instruments at Eskdalemuir Observatory.

Details of the site[3] and the history of its developing shelterbelts, up till 1958, are given elsewhere[4]. In the 1960s, the mature trees had become vulnerable to strong winds and, according to a forestry report of September 1967, even the more deeply planted shelterbelt on the east side and nearest to the thermometer site was substantially impaired. The comments by Callendar[5] on Eskdalemuir shelter and extensive research on windbreaks[6,7] suggest that the changes at Eskdalemuir are sufficient to merit serious consideration in any temperature-trend studies.

Investigations[8] in 1957 showed that winds from the Glasgow and Newcastle areas could be identified as pollution carriers. Although surface concentrations of air pollution at Eskdalemuir are small, possible effects on temperature of the complex neighbouring urban meteorological regimes cannot be ruled out.

Between 1925 and 1935, the temperatures used are presumably the daily maxima and minima for periods terminating at midnight[9,10], while for the period 1957–67 it is understood that the maxima refer to the "day-time" period from 0900 to 2100 and the minima to the "night" period from 2100 to 0900. This change of procedure might account, at least partly, for the decreases of maxima in January and July and the increases of minima in April and October, in Table 2. Presumably, the two algebraic expressions used for Table 2 should read

$$\Delta F_A(T_A - T_1)/N \text{ and } \Delta T_A, F_{2(A)}/N$$

Up to the end of 1956, all available maximum and minimum temperatures were measured in a large louvred (photothermograph) hut[10], but from 1957 the published and computer-processed temperature extremes were obtained from a large louvred screen using standard thermometers.

Analysis difficulties arising from such changes may often be overcome by the study of "station histories", the use of long consecutive series of data[11] rather than relatively short and isolated periods and by comparison with other suitable stations.

Confirmation of the results might be obtained from stations in the same area, but both the weather-type–temperature relationship and its trends may well differ from region to region. For example, in Scotland the coldest surface wind directions in winter are north-westerly or northerly, whereas in south-east England the lowest mean temperatures occur with easterlies or north-easterlies. A change to higher sea temperatures may cause a significant increase of mean air temperature in Scotland with south-easterlies, while a similar but much smaller effect in south-east England might be masked by other factors, or the total change there might be made even negative.

It is questionable whether the "Lamb catalogue" (details of which are not stated and are not as yet generally available) of daily synoptic weather classification of the United Kingdom area is suitable for an investigation concerning a particular station. The range of wind variation will normally be in excess of 45° and in the British Isles such large sectors could include directions with vastly differing air-mass tracks.

The results are also more difficult to interpret if wind speed data are not used. For example, a decrease of westerlies if accompanied by a decrease of mean wind speed could appear as a temperature trend arising from different mixing depths both in the atmosphere and the hydrosphere.

Broad classifications of air masses (see, for example, Belasco[12]) may be expedient, but to include, within one weather-type, synoptic patterns which differ at some distance from the station under study and in recent synoptic history, is an unsound procedure on which to base the conclusion that Dzerdeevskii's hypothesis should be modified.

In some circumstances, even the time unit (one month) may prove too long for this type of analysis. October may seem to be the only month with significant weather-type differences between the two periods for both maximum and minimum temperatures because there are large differences in solar elevation, and so on, between the beginning and end of that month.

The two periods studied are each eleven years long, but the solar cycle does not seem to have been considered. It has been suggested[13] that the lower frequency of westerly types over Britain during 1931–60, compared with 1901–30 (refs. 13–15), is related to the higher level of solar activity or larger amplitude sunspot cycles in the later period. These changes of the general circulation and sunspot regime may be associated with changes of, for example, sea temperature and consequently of within-type temperature trends. Any such weather–sunspot relationship could thus be considered by using longer, consecutive series, as previously recommended.

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"Weather-type" Frequencies and Temperature Fluctuation: a Reply

Lawrence's information on possible factors affecting the temperature record at Eskdalemuir Observatory, which we had unfortunately overlooked, is appreciated. It is, however, difficult to find suitable neighbouring stations, with homogeneous data series in a conveniently available form. With respect to the possible shelterbelt effects, the tendency would be towards higher maxima and lower minima in 1957–67 than in 1925–35. In fact, for anticyclonic type, which should best reflect this influence, the maxima and minima increase or decrease together in all four months investigated.

We regret the typographic errors in the two algebraic expressions in the paper. We should also like to note that the expression given for a temperature change within type (ΔT) implies no frequency change. This component should properly be evaluated from $\Delta T_A F_{1(A)}/N$. The recalculated values reinforce the view that changes in type frequency are the dominant factor in the temperature changes in January only.

We should make clear that the study was based on the original Lamb catalogue[1]. The revised catalogue, made available to us by Lamb, is being used in our further work. The need for investigation of the local weather characteristics of the types and (in our opinion) for linking the types with a suitable index of upper airflow has been referred to elsewhere[2,3]. Nevertheless, we consider that any attempt to devise a special, "local" catalogue of types, and smaller wind sectors, would create more problems than it would solve. The extent to which any one of the Lamb types may differ, from one period to another, with respect to the synoptic history of the airstreams involved, is worth studying. We have assumed that, for moderate-sized samples, there would be no systematic differences caused by track directions.

The two periods we selected were related to the recent peak and trough in the frequency of westerly type. Our initial aim was to assess the significance of this decrease in westerly airflow because many of the changes in Britain's climate since the 1930s have mainly been attributed to this factor[4,5]. As we stated, examination of the various influences which may effect within-type changes will be made when studies for other stations (and other weather parameters) have been completed.

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Directional Correlation Studies of Anomalous Water in Carbonic Anhydrase

THE technique of γ-ray directional correlations has recently been applied to biological macromolecules in solution[1]. We report here the use of this technique to study internal fields of water associated with hydrated dry proteins. We chose the nuclide ^{133}Cs, which is formed by the electron capture decay of ^{133}Ba ($T_{\frac{1}{2}} = 7·3$ yr). The 356–81 keV γ-ray cascade in this nuclide involves an intermediate level of nuclear spin 5/2 and half life $T_{\frac{1}{2}} = 6·3$ ns, and has been studied previously[3-7]. The results show that $A_{44} \ll A_{22}$ in the directional correlation function and the coefficient A_{22} may be found by measuring the anisotropy.

A 100 μCi sample of ^{133}Ba in 6 M HCl (New England Nuclear) was dried over NaOH and KOH pellets in vacuum. The radioactive film was dissolved in a solution of bovine carbonic anhydrase (150 mg/25 ml. deionized water) yielding a solution of final *p*H 6·5 which was frozen with liquid nitrogen and lyophilized to constant weight. The freeze-dried samples were then adjusted to approximately 5 per cent and 20 per cent water content by exposure to 0·2 and 0·6 relative vapour pressures, respectively. The samples were placed in sealed glass cylinders (internal diameter 0·4 cm; length 8·0 cm). Measurements of enzymatic activities of these samples showed no change with respect to the original enzyme preparation.

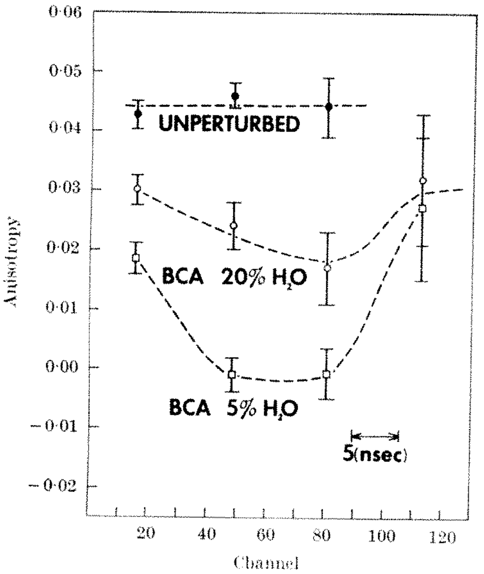

Fig. 1. Time dependence of anisotropy for 356–81 keV gamma cascade decay of ^{133}Cs in hydrated bovine carbonic anhydrase (BCA). Unperturbed anisotropy from ^{133}Cs in aqueous 6 N HCl solution.

The time dependent anisotropy of the 356–81 keV gamma–gamma cascade was measured by use of a two-detector time-differential coincidence system. Calibration of the time-to-amplitude converter was achieved by using known delay lines. A coincidence time resolution of 10 ns full width half maximum was measured by using annihilation radiation from the positron decay of ^{22}Na. The accidental coincidence rate was measured by using two isolated sources of ^{133}Ba. The anisotropy was found to be perturbed over a 40 ns time interval for both hydrated protein samples. The results (Fig. 1) are uncorrected for finite solid angle and are the result of weighted averages over the time resolution of the coincidence system.

The coefficient A_{22} was measured by performing an unperturbed directional correlation experiment. An 80 μCi sample of ^{133}Ba in aqueous 6 M HCl was used to minimize hydrolysis. Brownian motion in the solution eliminates the effects of perturbing fields and the unperturbed coefficient A_{22} may be measured. The time dependence of the anisotropy was measured as in the perturbed experiments. The result is shown in Fig. 1 and indicates no perturbations over time delays of 20 ns. The coefficient A_{22}, when corrected for finite solid angle, is found to be $A_{22} = 0·035 \pm 0·002$, in fair agreement with previously published results[3-7].

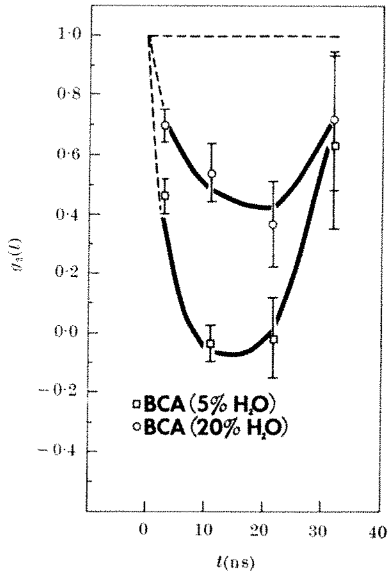

Fig. 2. Experimental perturbation factors with theoretical fit, for 356–81 keV gamma cascade decay of ^{133}Cs in hydrated bovine carbonic anhydrase (BCA).

The time dependent perturbation factors for both carbonic anhydrase samples are shown in Fig. 2. For the 5 per cent hydrated sample the behaviour of $g_2(t)$ indicates the presence of a static electric quadrupole interaction. The theoretical perturbation factor for such an interaction has been calculated[8] for an intermediate nuclear level spin of 5/2. A least-squares fit with theoretical curves corrected for finite time resolution shows the perturbation in the 5 per cent hydrated source to be caused by an axially symmetric electric quadrupole interaction which corresponds to an interaction-frequency of $\omega_0 = (2·09 \pm 0·04) \times 10^8$/s. This represents an interaction strength of $|Q\nabla E| = (9·14 \pm 0·18) \times 10^{-7}$ V. Because static fields are not expected in an aqueous dilute solution, it seems likely that the ^{133}Ba ions are occupying definite sites of axial symmetry in the carbonic anhydrase molecules. The data further indicate that all ^{133}Ba ions occupy equivalent sites, for the existence of several different interaction strengths would result in a damping of the oscillatory behaviour[8] of the perturbation factor: no such damping occurs.

For the 20 per cent hydrated carbonic anhydrase sample the behaviour of $g_2(t)$ again indicates the presence of a static interaction. At a time delay of 31 ns the directional correlation is indeed close to its unperturbed value. A comparison with the perturbation factor for the 5 per cent hydrated sample of carbonic anhydrase suggests the presence of a time dependent interaction which is seen by a certain fraction of the ^{133}Ba ions while the remainder occupy sites in the protein molecule and see only a pure static electric quadrupole interaction. If a fraction n_1 are influenced by static fields and a fraction n_2 by time dependent fields, the total perturbation factor will be given by

$$g_2(t) = n_1 g_{2s}(t) + n_2 \, e^{-\lambda_2 t} \qquad (1)$$

where $n_1+n_2=1$ and both $g_{2S}(t)$ and $e^{-\lambda_2 t}$ are corrected for finite time resolution. The static perturbation factor g_{2S} is known from the 5 per cent hydrated source. Equation (1) was solved numerically for several values of n_1, n_2 and λ_2. A best fit of the data gives $n_1=0{\cdot}45$, $n_2=0{\cdot}55$ and $\lambda_2=(8{\cdot}20\pm1{\cdot}00)\times10^6$ s^{-1}.

If the fraction n_1 of the ^{133}Ba ions are attached to the carbonic anhydrase molecules, then the fraction n_2 must be in the water associated with the molecules. If the water is normal, with a correlation time $\tau_c\simeq10^{-11}$ s, then time dependent interactions should not be observable. If the water is anomalous (that is, ordered) with a rotational correlation time much larger than 10^{-11} s, time dependent interactions become possible due either to fluctuating fields in the water or to the propagation of the electron hole following the electron capture decay of ^{133}Ba. It is suggested that the fraction n_2 of the ^{133}Ba ions are in the anomalous water associated with the carbonic anhydrase molecules. A relaxation time for this environment may be obtained from λ_2. We find $\tau=1/\lambda_2=(1{\cdot}22\pm0{\cdot}15)\times10^{-7}$ s, which indicates that the water associated with carbonic anhydrase molecules is highly ordered.

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Luminescence of Photoproducts at Defects in a Doped Inert Matrix

THE importance of defects in solid state reactions is well known[1] and the possibility of the direct observation of photochemical reactions at defects is of interest. Cohen *et al.*[2] irradiated a single crystal of acenaphthylene with ultraviolet light and produced a photodimer product located at the crystal defects. Unfortunately, host and reactant are the same species and the question remained whether the photoproduct migrated to the defects or was formed at the defects.

We have also observed photoproducts associated with crystal defects, but we used a two-component system which differs in important respects from that of Cohen *et al.* They used a host crystal entirely composed of photoactive aromatic molecules, which absorb ultraviolet light, react and luminesce. The host material we used is cyclododecane (CDD), which is transparent and photoinert. The CDD is deliberately doped with photoactive aromatic molecules and the distribution of guest aromatic molecules has been investigated[3] for single crystals grown by the Bridgman–Stockberger method (Fig. 1). D. J. M. and Mathur[3] have shown that, for phenanthrene concentrations up to 5×10^4 M, the distribution is in cellular patterns and probably monomolecular. Phosphorescence lifetime measurements[4] demonstrate a monomolecular dispersion below these concentrations. At higher concentrations precipitation occurred at defects. A CDD lattice vacancy is at least $8{\cdot}5\times8{\cdot}5\times4$ Å and can just about accommodate a phenanthrene molecule in a diagonal aspect, the latter having dimensions approximately $11{\cdot}6\times8\times3{\cdot}2$ Å.

We prepared samples by outgassing a mixture of CDD and phenanthrene in a tube under vacuum. The tube was sealed, the mixture molten and re-solidified. The tube was then broken to yield a gas-free solid sample. This was sandwiched between two quartz slips on a hotplate, gently heated until the CDD melted and some liquid was ejected at the sides. The centre portion, which remained, was cooled slowly. Several single crystal regions were observed to form.

On irradiating 5×10^{-3} M samples an induction period of 5 min was followed by a growth of the fluorescence

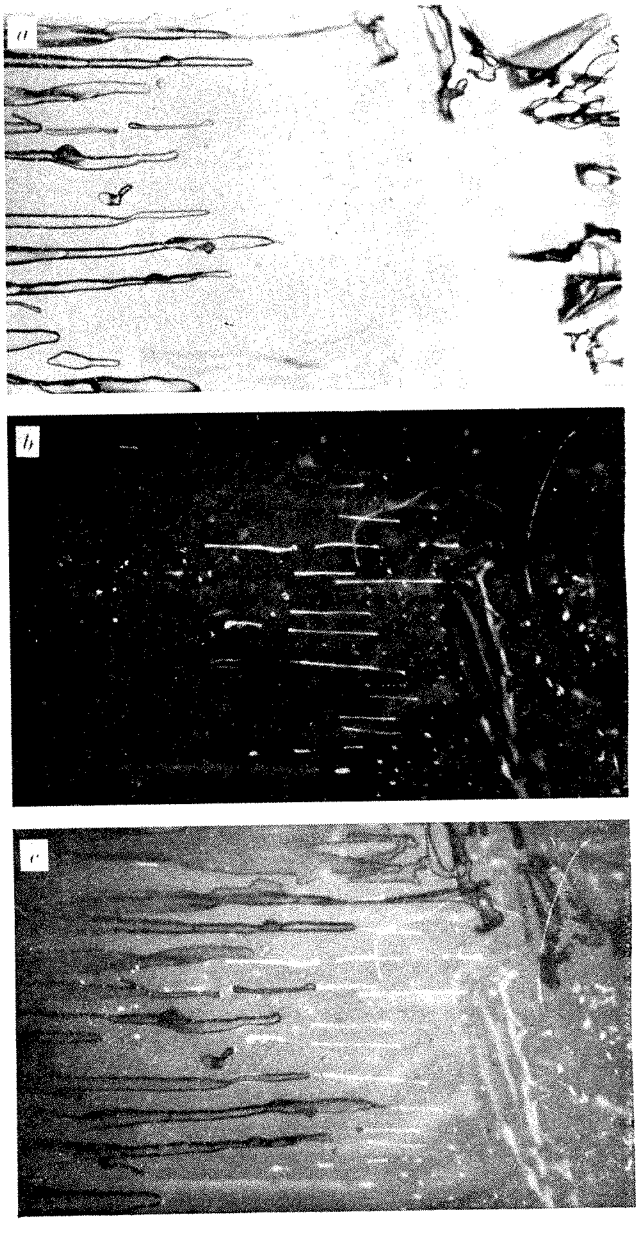

Fig. 1. Low power photomicrograph of phenanthrene doped cyclododecane crystal. *a*, Under transmitted white light; *b*, under 365 nm radiation from beneath sample; *c*, under mixed ultraviolet irradiation and transmitted light.

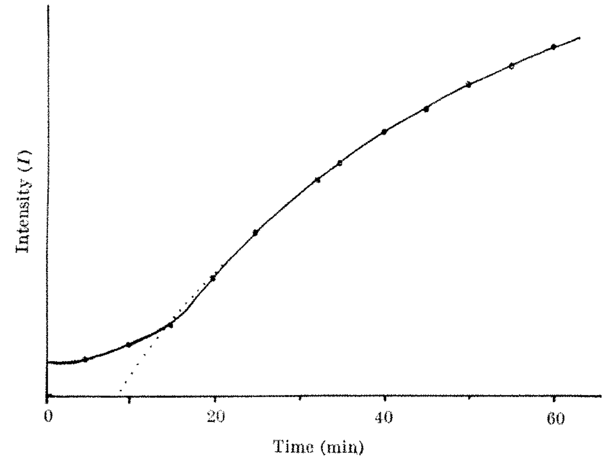

Fig. 2. Relative fluorescent intensity (I) of defect material as a function of time (t). The dots represent the closest fit of an exponential curve.

intensity, I, to a maximum I_0 (Fig. 2) according to the relationship

$$I = I_0\,[1 - \exp(-kt)]$$

where t is the time elapsed after the induction period. We measured the intensity at 450 nm, which is the band maximum (Fig. 3) for the fluorescence. The phenanthrene band was determined by overdoping until precipitation occurred and also using small phenanthrene crystals; this peaks at 435 nm (Fig. 3). Observations at 435 nm showed an initial rapid decrease during the induction period. An initial reaction with impurity, probably dissolved oxygen, until the latter is depleted is suggested. The exponential term suggests a first order decay of reactant, so that polymerization is not likely to an extent beyond dimerization. We attempted to characterize the reaction product by thin-layer chromatography. Spots were prepared of pure phenanthrene; doped CDD not irradiated; and doped CDD irradiated, each dissolved in 3-methylpentane, using n-heptane as the solvent. The chromatogram was run for 10 cm and the result is shown in Fig. 4. The positions of the phenanthrene and of the CDD showed, under 253·7 nm light, as a blue fluorescence and as a whitish patch respectively. The spot corresponding to phenanthrene showed no signs of impurity and that corresponding to non-irradiated doped CDD showed no signs of a third component. The irradiated sample showed both CDD and phenanthrene, as well as a new component which appeared as a streak between the baseline and the phenanthrene spot. The R_F value of the photoproduct, being lower than phenanthrene, suggests that it may be a larger molecule than phenanthrene or possibly a polar oxidation product.

The advantages of the system we used derive from the fact that only the guest and its photoproducts are photoactive. Thus we are able to use transmission microscopy techniques and we can observe the photoproduct by its own luminescence unimpeded by host absorption or emission.

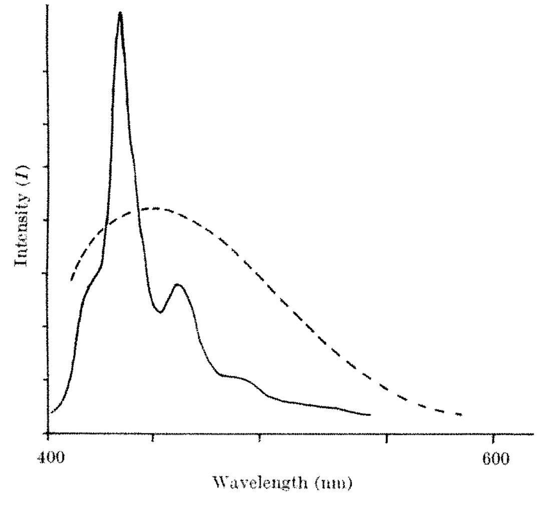

Fig. 3. Emission spectra observed through the microscope. - - - -, Emission from defect material: ——, emission from precipitated phenanthrene in a concentrated sample.

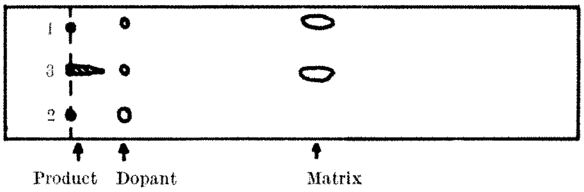

Fig. 4. Schematic representation of a chromatogram, viewed under ultraviolet light. The trace obtained from non-irradiated phenanthrene sample (1) shows two components. The trace for phenanthrene only (2) shows one component. The trace for an irradiated sample (3) shows new components as a streak.

The imperfections we observed included planes and cracks which run perpendicular to the plane of the quartz plates. The cracks developed at the termini of the defect planes as the crystal grew. We also observed these features in a pure, undoped, CDD sample using interference microscopy. The defect planes probably arise from the release of internal stresses set up by contraction as the material solidifies. The planes and cracks are unequivalent, not only in their morphology but in their fluorescence behaviour. We cannot dismiss these distinctions. It is unreasonable to expect photoproduct to migrate to defect planes and not to cracks. We may expect, on the other hand, that the phenanthrene is aggregated in defect planes and probably also on the cracks. We can assume that some traces of oxygen are present in the crystal which are exhausted in the planes during the induction period. Sufficient oxygen may be present in the free spaces in the cracks so that photooxidation occurs there throughout the irradiation period.

The appearance of the planes is regular and flat, contrasting with the cracks. We believe these to be defect planes in which the phenanthrene may be orientated in thin layers. These planes must, in any event, be dense enough to inhibit oxygen diffusion. Ferguson[5] has shown that excimer emission occurs in cyclohexane crystals at 77 K and considers that the crystal host provides orientation conditions which favour excimer formation.

Recalling that the reaction could be considered as first order, we may envisage that after the absorption of a photon by one of the phenanthrene molecules it forms an excimer which reacts, or alternatively it reacts directly to form a dimer. This can occur between adjacent pairs of molecules in a monolayer until it is depleted.

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Observation of Substructures in Photoinert Organic Single Crystals doped with Photoactive Impurities

THE photoexcitation properties of organic crystals have generally been studied for pure or mixed aromatic single crystals. In these cases the host is photoactive[1–3]. The substructures of such molecular crystals are not as well known as in the cases of ionic[4] and metallic crystals[5], although such structures may well influence the properties involved in photoexcitation. D. J. M. and Irons[6] used cyclododecane (CDD) single crystals as the photoinert host for phosphorescence lifetime studies of the photoexcited triplet state of dissolved aromatic impurities. Lifetime variations were found and these were attributed to various states of aggregation of the photoactive solute species in the single crystal host. The tendency to aggregate was correlated to lattice mismatch between host and dopant molecular dimensions. The present work establishes the presence of aggregates in doped single crystals of CDD (cyclododecane) by direct observation.

Doped crystals of organic materials grown by the Bridgman–Stockberger method[7,8] contain large numbers of imperfections[9,10]. During the unidirectional freezing of the two component system, the solute distribution ahead of the solid/liquid interface is, of necessity, not uniform. Instead, a concentration gradient is formed which violates the condition for the stability of planar solid/liquid interfaces. This leads to constitutional supercooling and impurity substructures[11,12]. We have indeed observed, in CDD crystals, the pox and cellular structures commonly found in constitutional supercooling and have also studied the effect of the nature and amount of the solute content on the microstructure of a CDD single crystal. The CDD was purified, and doped crystals grown as described elsewhere[6]. The aromatic phosphor impurities were added in weighed amounts, to yield concentrations of 10^{-2} M in the melt with respect to the host material. The concentrations which result in the crystal were lower by one or two orders of magnitude. The grown crystal was sectioned parallel to the growth direction. We used a "chemical" saw which was dipped every now and then in a suitable solvent such as carbon tetrachloride. A smooth crystal surface was obtained for optical microscope examination by grinding on a flat glass paper. This was followed by further polishing using a tissue paper, which was soaked in CCl_4 and placed on a flat glass plate. The crystal was then dipped in CCl_4 for 5 s and washed briefly with absolute alcohol. Finally, the crystal was etched with absolute alcohol for 3–5 min and subsequently examined by standard optical methods.

We have observed network structures characteristic of constitutional supercooling. Fig. 1 shows a transmission photomicrograph of CDD doped with phenanthrene (melt concentration 10^{-2} M, concentration in crystal 5×10^{-4} M); the growth direction is parallel to the plane of the photomicrograph. The photograph shows the cell nodes, preferentially etched, which resulted from constitutional supercooling. The nodes are reliable indications of the solute distribution. We confirmed this through the bulk of the crystal by observations of the same sample using fluorescence microscopy. Fluorescent spots, attributable to phenanthrene, were seen distributed through the bulk in similar cellular patterns. These latter observations were made possible by the transparency of the host crystal. While advantage can be taken of transparent hosts for ionic species[4] and for infrared transparent[13] crystals, this is not possible in the case of aromatic single crystals where the whole of the matrix will absorb ultraviolet. This problem has been overcome in an interesting way by Cohen, Ron, Schmidt and Thomas[14], who produced photodimers in a thin aromatic single crystal, presumably at dislocations, and who observed the resulting fluorescence at dislocations. This last method provides, to our knowledge, the only other method to the one we report here for the observation of dislocation networks in a bulk organic crystal. A similar effect, using doped photoinert CDD crystals, has also been reported and characterized by D. J. M. and Miles[15].

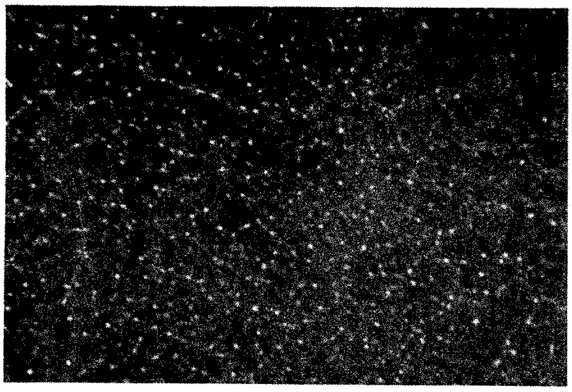

Fig. 1. Photomicrograph of phenanthrene doped CDD (×60) showing cell nodes, preferentially etched.

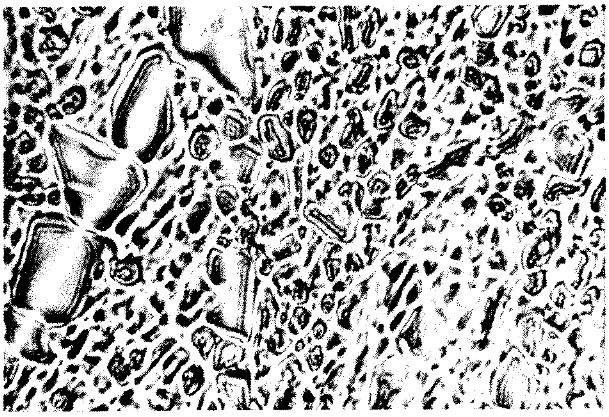

Fig. 2. Photomicrograph of triphenylene doped CDD (×400) showing a microsegregation "necklace" across the upper left hand corner.

We have studied the constitutional supercooling process by varying the molecular parameters of the solute, using the same growth and concentration conditions as for Fig. 1. Fig. 2 shows a transmission photomicrograph for a triphenylene sample and, in this case, we see microsegregation of the triphenylene as well as a cellular structure. This microsegregation behaviour was not seen in any of our phenanthrene samples at the same concentration. The microsegregation evidently arises from lattice mismatch as predicted by D. J. M. and Irons[6], while mismatch between phenanthrene and CDD does not occur. When, however, the concentration of the phenanthrene was increased to 5×10^{-2} M in the melt, well characterized precipitation did occur. The precipitated microcrystals of solute were observed by transmission microscopy and also by means of their fluorescence under ultraviolet excitation. Using a melt concentration of 10^{-2} M, which is the same as for Fig. 1 and Fig. 2, chrysene doped CDD gave precipitates only, and we have not seen cellular structures for chrysene in CDD crystals.

We have observed that cellular structures occur from the aromatic dopants we have studied only where solubility of the dopant in the host is assured by matching molecular dimensions. An apparent exception is the case of benzil which also yields cellular structures and, as noted by D. J. M. and Irons, the benzil molecule dimensions can be

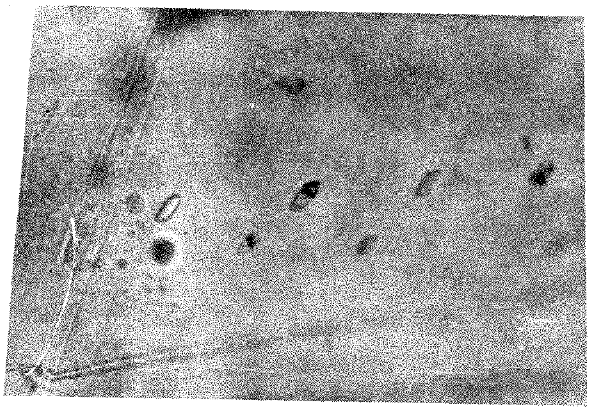

Fig. 3. Photomicrograph of phenanthrene doped CDD (5×10^{-2} M in the melt) showing precipitation of phenanthrene.

matched by a pair of CDD molecules. The tendency to precipitate appears to be related to the lattice mismatch through the segregation coefficient. The tendency to precipitate increased in the following order: phenanthrene < carbazole < triphenylene < chrysene. This order corresponds to increasing lattice mismatch. We expect this order to be inversely related to the segregation coefficient and preliminary work indicates that this is so. The similarity in the cellular structures observed in CDD organic crystals to those seen in alloys is not surprising, but merits comment. In both cases the passage from a soluble to an insoluble condition will depend on physical factors, namely, the solubility and growth parameters. The hexagonal appearance of the cells corresponds to a close packed arrangement consistent with a maximum ratio of cellular content to cellular surface.

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BIOLOGICAL SCIENCES

Timing of Neuroblast Multiplication in Developing Human Brain

As a result of experimental studies using growing rats[1] and pigs[2], it has been established that the developing brain is most vulnerable to restrictions such as undernutrition at the time of its fastest growth. Restriction at this time need not be severe to produce permanent deficits. This vulnerable period, the so-called brain "growth spurt", varies considerably between species only in its timing in relation to birth[1]. It has thus become desirable to define it for the human species and we report here part of an extensive quantitative study of human brain growth undertaken to this end.

Brains were obtained from twenty-four human embryos resulting from therapeutic abortion as well as from a series of stillbirths and perinatal deaths. The abortion material from 10 to 22 weeks of gestation has all been considered normal, and its gestational age estimated from the menstrual history and the foetal size. The later autopsy material, comprising fifty-two cases, was selected on the basis of the birth weight being within one standard deviation of the mean for the gestational age[3] and having no gross cerebral or other pathology. Total cell numbers were estimated by chemical analysis[4] of DNA–P in the forebrain, cerebellum and brain stem, and the whole brain values calculated as the sum. The relatively small number of tetraploid cells in the normal brain[5] were not considered to influence the assumption that total tissue DNA represented total numbers of cells. It was also established, by the use of freshly killed rat brain, that the quantity of DNA was unaffected by the inevitable delay between death and the collection of the human material.

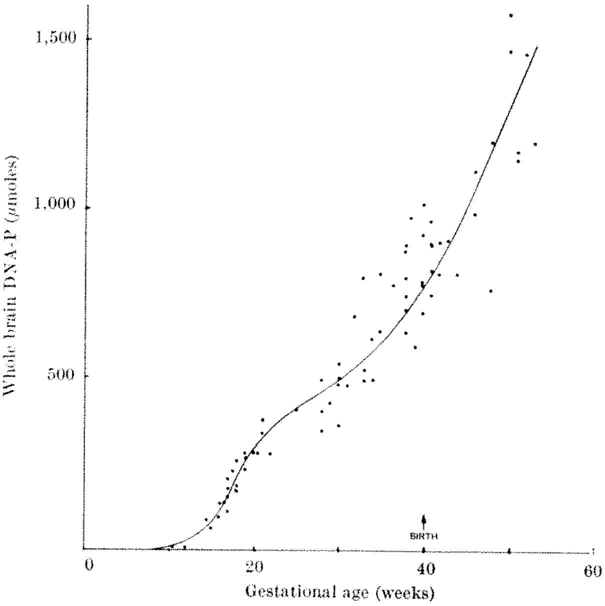

Fig. 1. The increase of whole brain DNA in developing human brain.

Fig. 1 illustrates the new finding that there are two major periods of cellular multiplication in human brain: one from 15–20 weeks of gestation and one which commences at about 25 weeks of gestation. This second period probably ends in the second year of postnatal life. The previous failure[6,7] to detect the earlier period can be ascribed to an insufficient number of cases for analysis at the relevant gestational age. The failure to detect the same events in studies of animal brain development presumably results partly from the difficulties imposed by a much faster developmental time scale in small laboratory species and partly from a failure to collect sufficient specimens at an early enough age.

Fig. 2 shows the result of plotting the logarithm of the DNA–P value against the age, and treating the samples between 10 and 20 weeks separately from the later material. It is clear that two distinct phases of cell division exist, each with different exponential characteristics.

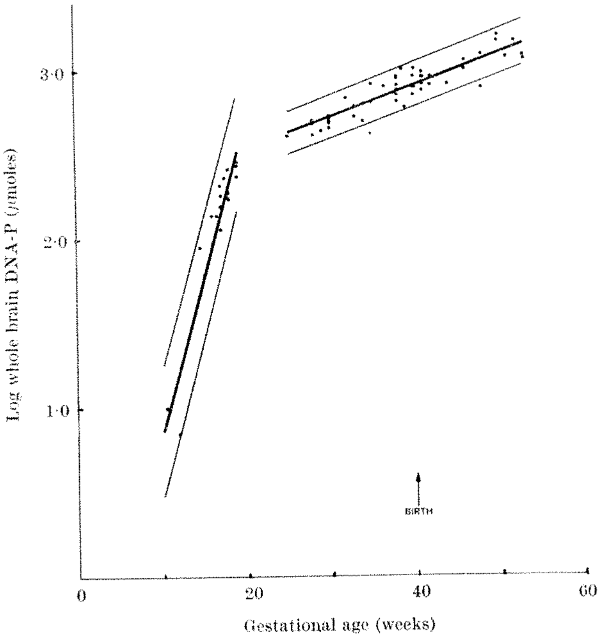

Fig. 2. The logarithm of whole brain DNA in developing human brain. Regression lines and their 95 per cent tolerance limits have been calculated to illustrate the two phases of cell multiplication (see text). First phase: Log whole brain DNA–P_{10-19} = − 0·9830 + 0·1845 × age. Tolerance limits ±0·3886. Second phase: Log whole brain DNA–P_{25-33} = 2·1555 + 0·0188 × age. Tolerance limits ±0·1325.

It is well known that the adult number of neurones is achieved quite early in brain development, before the phase of glial cell multiplication begins[8]; and that glia in the adult brains of large species outnumber neurones by several fold[9]. From this it seems likely that the first phase of multiplication reported here, occurring from 15–20 weeks of gestation, represents the period of neuroblast proliferation. The same phenomenon has also been found in the increase of DNA in the human forebrain and cerebellum. In both regions the timing is identical to that shown in Figs. 1 and 2. The second phase must predominantly represent glial division.

Undernutrition restricting growth at the "vulnerable period" defined here produces an ultimate cell deficit in the adult which is probably mainly glial. Whether earlier restriction applied during this newly discovered first period of DNA increment would lead to greater behavioural impairment is now being investigated.

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Space–Time Transformation in the Frog Cerebellum through an Intrinsic Tapped Delay-line

THE cerebellum is interesting for the arrangement of its neurones in a symmetrical three-dimensional lattice, with only two classes of input fibres and one of output fibres[1]. This arrangement led Braitenberg[2] to postulate a set of logic operations that the cerebellar cortex might perform. One postulate was that, by virtue of the distribution of the "output" neurones (Purkinje cells) at regularly spaced intervals along beams of parallel fibres (Fig. 1*A*), the cerebellum might function as a type of clock, transforming an event that occurred at some peripheral spatial location into a temporal event. A central assumption of this hypothesis is that the time of firing of a Purkinje cell is determined by the conduction time of afferent impulses travelling in parallel fibres from some reference point on the beam of parallel fibres on which it lies to the Purkinje cell. If this assumption is correct the parallel fibre–Purkinje cell system would constitute a tapped delay-line[2–4]. If the reference point is the position of another Purkinje cell along the beam, then a corollary is that the time between the firing of the two cells must be equal to the conduction time of the segment of the beam connecting them.

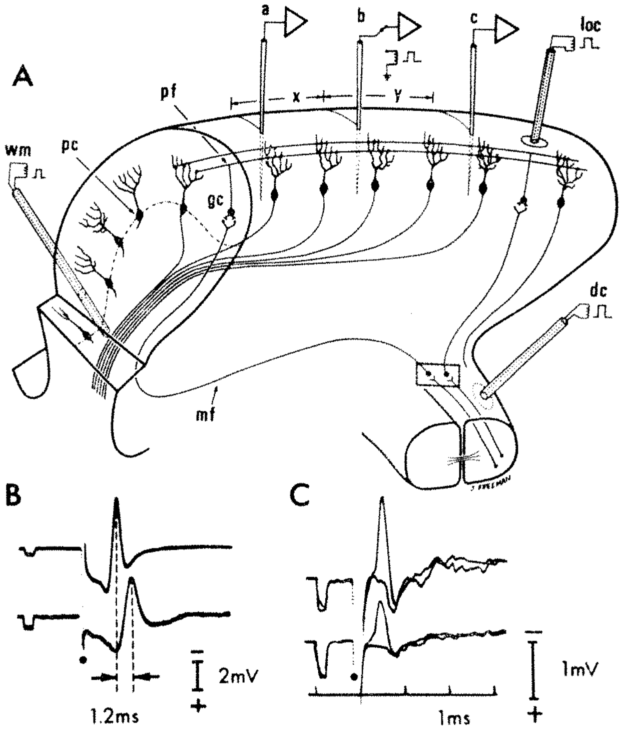

Fig. 1. *A*, Diagram of frog cerebellum and experimental arrangement described in text. pc, Purkinje cell; gc, granule cell; pf, parallel fibre; mf, mossy fibre. *B*, Extracellular field potentials evoked by surface stimulation recorded simultaneously at two different points 250 μm apart on the same beam of parallel fibres. Conduction time of the parallel fibres between the two electrodes is indicated by arrows. *C*, Simultaneous extracellular recording of a pair of Purkinje cells activated antidromically. Two superimposed sweeps (stimulus intensity greater and less than threshold) revealing an all or none response.

Because time is a crucial parameter in the coordination of muscular activity—a presumed function of the cerebellum—Braitenberg's hypothesis has been of considerable interest, although it has never been tested. One apparent difficulty is that the mossy fibres branch extensively when they enter the cerebellar cortex[5]. If the branching were such that two Purkinje cells lying on the same beam of parallel fibres were activated by parallel fibres arising at multiple points randomly distributed between them, then the delay between their firing would vary randomly, and Braitenberg's hypothesis would fail. This motion was tested by computing the degree of correlation between the firing of two (and sometimes three) simultaneously recorded frog Purkinje cells lying on the same beam of parallel fibres.

Bullfrogs (*Rana catesbiana*) were immobilized with 4 mg/kg of *d*-tubo-curare, the tissues overlying the skull were anaesthetized with 'Procaine' and the cerebellum was exposed. The animals were placed on a small rotatable platform, to provide natural vestibular stimulation. The trajectory of a beam of parallel fibres was determined by activating the beam with a surface stimulus applied near one side of the cerebellum (Fig. 1*A*, "loc"), and tracking along it with a pair (or triplet) of microelectrodes (micropipettes filled with 4 M NaCl for extracellular recording *a*, *b* and *c*, Fig. 1*A*). Electrode positions were adjusted individually until the field potentials generated in response to the parallel fibre stimulus were maximal. In thirty-six frogs the parallel fibre conduction velocity *v* (computed by dividing the distance *x* between electrodes by the time between the peak negativity of the field potentials[6] (Fig. 1*B*)) was 20 ± 2 cm/s. This value was quite uniform over the width of the cerebellum for a given frog. Purkinje cells were identified by being activated antidromically (by stimulation of their axons with an electrode placed in the cerebellar white matter (Fig. 1*A*, "wm"))[7]. All or none antidromic responses from two simultaneously recorded Purkinje cells are shown in Fig. 1*C*.

To determine the degree of correlation between two cells, a LINC-8 computer was used to compute first order serial correlation[4] ("cross interval"[8]) histograms, that is the distributions of times from an action potential produced by one cell, to the first preceding and following action potential produced by the other cell. This analysis can reveal some additional important characteristics of the mossy and climbing fibre inputs to the cerebellar cortex. For example, consider two Purkinje cells separated by a distance *x*: because of their uniform conduction velocity *v*, parallel fibres which arise outside the zone *x* will tend to cause them to fire at a single, fixed interval, *x*/*v*, producing a peak at the same interval on the histogram. A pair of peaks, symmetrical about the origin, will be produced if parallel fibres arising on both sides of the zone *x* are activated. On the other hand, parallel fibres arising between the cells tend to cause them to fire at an interval proportional to the relative distance from the parallel fibre branch points to the two cells. The positions of any peaks on the corresponding histograms therefore reveal the locus of predominant parallel fibre activity. Finally, if (*a*) parallel fibres synapsed with one, but not both cells, or fired asynchronously in response to the activation of mossy fibres, or (*b*) the two cells were activated predominantly by their separate climbing fibre inputs rather than by their shared parallel fibre inputs, they might fire in an uncorrelated manner, producing a histogram which decayed exponentially from a maximum value at the origin[4].

Forty-eight pairs of Purkinje cells were analysed. The records on one pair, 150 μm apart, are shown in Fig. 2. The sharp peak at 0·75 ms in response to parallel fibre stimulation (Fig. 2*A*) shows clearly the sequential activation of the two cells as the volley travelled from one to the other. During angular acceleration both cells responded with a sustained burst of spikes (Fig. 2*C*), and, as shown by the prominent peak at 0·75 ms in Fig. 2*B*, they fired in the same sequence and at the same time intervals which would be produced by unidirectional activation of the beam of parallel fibres connecting them. Similar correlated firing was observed in thirteen pairs of cells in response to angular acceleration, and in forty-one pairs in response to stimulation of the spinal cord (*d*, *e* in Fig. 1*A*), and of the sciatic and radial nerves.

Two types of experiments proved that the correlation in times of firing was mediated by the parallel fibres connecting the cells. A current source-density analysis[9] revealed that the parallel fibres were activated synchronously by the same stimuli that caused the cells to fire in sequence; whereas blocking the conduction of parallel fibres between the cells (by a collision technique[4]) abolished the correlation.

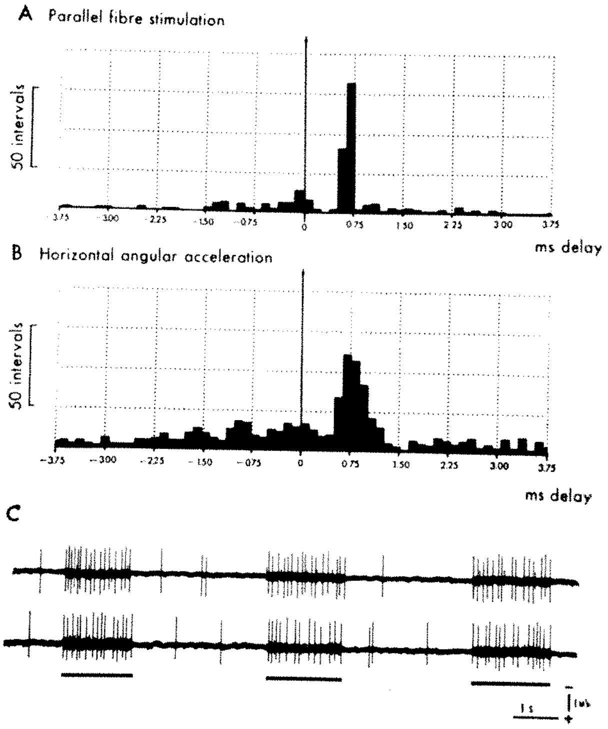

Fig. 2. Correlation between times of firing of two Purkinje cells on the same beam of parallel fibres. *A* and *B*, First order serial correlation histograms computed during stimulation of the beam of parallel fibre connecting the two cells (100 stimuli, 1 stimulus/2 s) and during horizontal angular acceleration (approximately $2^{\circ}/s^2$), respectively. Peaks at 0·75 ms in both records show that the firing of the two cells was correlated: cell *X* (lower record in *C*) tended to fire at a fixed interval after cell *Y* (upper record in *C*) equal to the conduction time along the beam of parallel fibres connecting them. *C*, Single unit records of the two simultaneously recorded Purkinje cells during horizontal angular acceleration (indicated by the horizontal line).

The time between the firing of two Purkinje cells increased linearly with increasing distance between the cells. By contrast, in only one pair of cells was correlated firing observed during spontaneous activity (these cells were only 50 μm apart); and in fifteen pairs of cells which lay "off beam" no correlation was found.

These results suggest that in the frog (1) geometrical specificity is preserved in the distribution of mossy fibre terminals, in spite of their extensive branching; (2) a substantial number of the parallel fibres connecting a row of Purkinje cells fire in synchrony in response to some natural and electrical stimuli; (3) during this type of stimulation, the Purkinje cells fire predominantly in response to their parallel fibre rather than to their climbing fibre input; (4) peripheral events are represented both temporally and spatially in the cerebellar cortex, in the sense that simultaneous events, or aspects of a single event, in the external world can be coded as a temporal sequence of activity in a discrete population of Purkinje cells; conversely, an external event occurring at a particular time can be coded as a spatial sequence of Purkinje cell activity, and (5) in response to some natural stimuli, the parallel fibre system functions as a physiological tapped delay-line, distributing excitation in a precise temporal sequence along rows of Purkinje cells.

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Effect of Pretreatment with Oestradiol, Progesterone and DOPA on Monoamine Oxidase Activity in the Rat

THE enzyme monoamine oxidase (MAO) (EC 1.4.3.4), which is widely distributed in the animal kingdom, is probably involved in the *in vivo* inactivation of a number of biologically active monoamines of physiological importance although its precise function has yet to be defined. Its putative role in the pathogenesis of mental disease has tended to focus interest on the brain in recent years, rather to the exclusion of other anatomical sites. J. S. *et al.*[1], however, have lately drawn attention to a variant of this enzyme present in human endometrium which undergoes sharp changes in activity during the different phases of the menstrual cycle. Although there is some evidence to suggest that such cyclical variations do not occur outside the female genital tract in the human[2], small fluctuations of enzyme activity have been noted in several rat and guinea-pig tissues during the oestrus cycle[3-7]. The most likely explanation for all these changes must presumably be based on alterations in hormonal levels, and to investigate this possibility we sought to influence the MAO activity of rat tissues by pretreating the animal with oestradiol and progesterone. Although hormones such as these may mediate the observed changes, the available data do not shed any light on how direct is the cause and effect relationship. There may be intermediate stages in the train of events leading to alterations in enzyme activity. A number of reports[8,9] point to variations in tissue concentration of amine substrates of MAO during the phases of the oestrus cycle. It is possible therefore that physiologically occurring cyclical increases in MAO activity might represent an adaptive response to increased availability of substrate. We have attempted to evaluate this possibility indirectly by treating rats before enzyme assay with DOPA which leads to increased tissue levels of the MAO substrate, dopamine.

Twenty-four 120–150 g female white Wistar rats were divided into four groups of six animals; one group was injected subcutaneously on three consecutive days with 3·3 mg/kg progesterone and another with 3·3 mg/kg oestradiol for a similar period of time. A third received 100, 150, 200 and 300 mg/kg DL-DOPA intraperitoneally on four consecutive days while the remaining group was given 0·9 per cent (w/v) NaCl solution, 0·25 ml. subcutaneously and 0·75 ml. intraperitoneally, for three consecutive days. On the day after completion of treatment, animals were killed by dislocation of the neck. Tissues were removed, freed from connective tissue, weighed and stored at −20° C. They were homogenized in ice-cold 0·3 M sucrose for 1 min and centrifuged at 600*g* for 15 min to sediment the cell debris. The supernatant was spun at 17,000*g* for 30 min to sediment the mitochondrial fraction. Finally, the microsomal fraction was isolated by centrifugation at 56,000*g* for 60 min. All fractionations were carried out at 4° C. Suitable suspensions were made in phosphate buffer (0·05 M, pH 7·4) and assayed for MAO activity using kynuramine as substrate[10]. Protein nitrogen was determined[11] using crystalline bovine serum albumin as a standard. MAO activity was expressed as ng 4-hydroxyquinoline formed/min incubation/mg protein nitrogen.

Table 1. EFFECT OF OESTRADIOL, PROGESTERONE AND DL-DOPA ON RAT TISSUE MAO

	Control	Oestradiol	Progesterone	DL-DOPA
Uterus	75·0 ± 6·0	57·7 ± 6·0†	125·9 ± 8·1†	131·3 ± 10·6‡
Ovary	146·0 ± 16·0	127·3 ± 12·1	161·2 ± 23·1	131·0 ± 20·2
Heart	155·5 ± 9·8	177·5 ± 16·3	169·9 ± 14·2	123·5 ± 14·3
Brain	196·3 ± 8·2	197·6 ± 8·8	196·2 ± 16·9	190·6 ± 13·1
Liver	998·7 ± 87·5	723·3 ± 42·9*	900·9 ± 30·2	921·5 ± 32·0
Sub-maxillary gland	56·9 ± 3·5	52·7 ± 2·7	54·3 ± 2·3	53·4 ± 3·6

Activities are expressed as ng kynuramine deaminated/min incubation/mg protein ± *S.E.* See text for description of the experimental procedure.

* $P < 0{\cdot}02$. † $P < 0{\cdot}01$. ‡ $P < 0{\cdot}001$.

Table 1 illustrates total MAO activity (that is, the sum of activities of cell debris, mitochondrial, microsomal and supernatant fractions) of various tissues. After oestrogen pretreatment there was a significant fall in activity in uterus and liver. Progesterone pretreatment, however, caused a significant increase in the uterus alone, and other tissues were quite unaffected. A similar specific increase in uterine MAO activity occurred after DL-DOPA treatment. Parallel changes were noted when activities were expressed per g wet weight of tissue. The general pattern of alteration in uterine enzyme activity was mirrored by all subcellular fractions (Fig. 1); the patterns for other organs did not bring out any effect of drug treatment.

Four uteri from each group were examined histochemically for MAO activity[12], as described by J. S. *et al.*[1]. Changes after oestradiol and progesterone pretreatment corresponded with the quantitative estimations (Fig. 2), whereas those after DOPA administration were less clear. MAO activity was localized mainly in the endometrium but a small constant level was also maintained in the myometrium.

The present observation that progesterone pretreatment brings about a specific increase in rat uterine MAO activity is in agreement with a hormonal explanation of our previous finding of an approximately ten-fold increase in human endometrial enzyme activity at about the

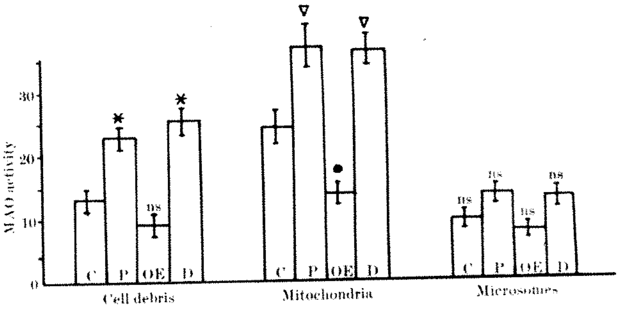

Fig. 1. Monoamine oxidase activity (expressed as ng 4-hydroxyquinoline formed/min incubation/mg protein nitrogen) of the various subcellular fractions of rat uterus. Each column represents the mean of six observations ± *S.E.* and comparisons of significant differences are made between each drug treatment and control values. Activity in the supernatant was less than 1 per cent of the total and has not been included. C, Control; P, progesterone; OE, oestrogen; D, DL-DOPA; ns, not significant. * $= P < 0{\cdot}001$, ● $= P < 0{\cdot}005$ ∇ $= P < 0{\cdot}05$.

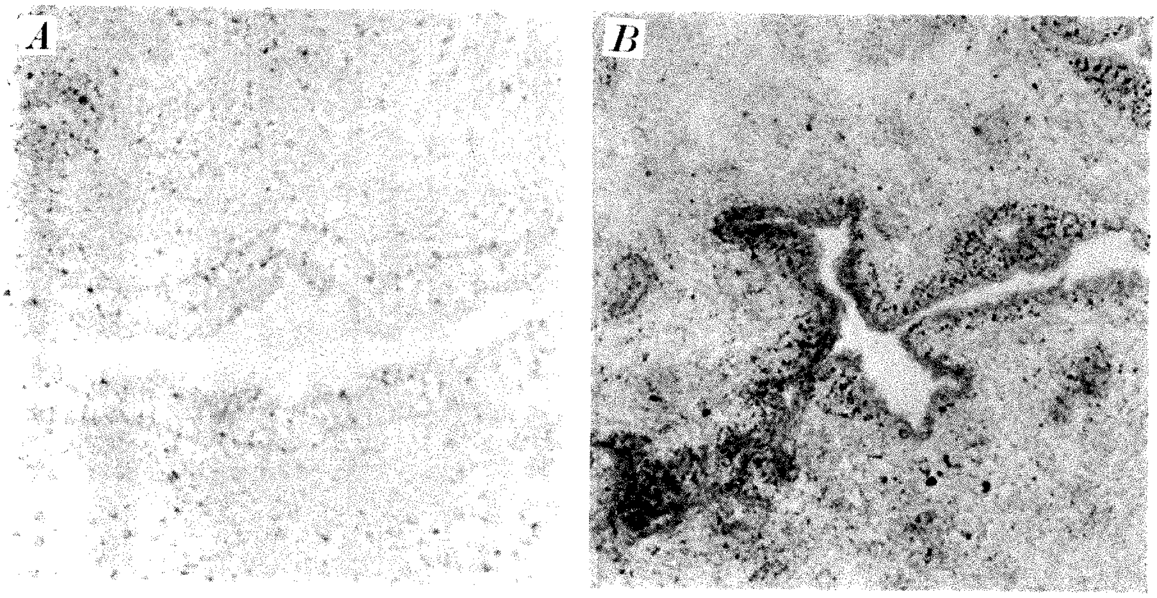

Fig. 2. *A*, Rat endometrium after oestrogen administration showing weak monoamine oxidase activity as scattered dye particles (nitro blue tetrazolium method × 200). *B*, Rat endometrium after progesterone administration showing strong monoamine oxidase activity. There is a diffuse dark (blue) staining of endometrium and some dye is present in fat droplets (nitro blue tetrazolium method × 200).

nineteenth to twenty-first day of the menstrual cycle[1] when plasma progesterone levels are known to be at their peak[13]. Further support is provided for this interpretation by Grant and J. P.-D.[14], who recently observed that administration of strongly progestational oral contraceptives causes a prolongation of the late secretory phase of the human endometrium accompanied by a corresponding increase in MAO activity. It may be that fluctuations of circulating hormone levels are not as marked during the oestrus cycle compared with the menstrual cycle, for rat uterine MAO undergoes no apparent change[1,3]. There have been several reports, however, of variations in MAO activity during the oestrus cycle in specific areas of rat and guinea-pig brain[3-6]; but if any such localized changes do exist, they were masked in our whole organ homogenate. Apart from the uterus, the only organ in which we could detect any change after drug administration was the liver in which, as with the uterus, MAO activity was reduced after oestrogen pretreatment. This finding is in line with similar data of Wurtman and Axelrod[15] and also correlates with a report that mouse liver MAO activity is lower during oestrus compared with pro-oestrus[7]. It is probably relevant in this connexion that cardiac MAO activity is lower in the female than the male rat[15,16]. Oestrogen dosage gave rise to an unequivocal decrease in rat uterine MAO and, although this finding might not have been predicted from the data of earlier workers[17-19], it agrees well with our previous observation[1] of comparatively low activity in the human endometrium during the non-secretory phase of the menstrual cycle.

At present, data are not available to show whether the amine concentration of the human uterus fluctuates during the menstrual cycle; in the rat uterus, however, endogenous noradrenaline concentrations are significantly raised during oestrus[8] and circulating adrenaline is accumulated more avidly during oestrus than dioestrus[9]. We have found that DOPA treatment, which increases tissue levels of dopamine[20], a good substrate of MAO[21,22], gives rise to increased uterine MAO activity either by induction of enzyme synthesis or by a more direct action on the catalytic mechanism. Whether the mechanisms of action of the drug treatments are in any way interconnected or have any physiological significance can only be decided by future experiment. One other point emerges: the MAO variant found in the uterus differs from other rat tissue MAO isoenzymes[23-25] in its sensitivity to hormones and is the subject of further intensive study. Whether progesterone which potentiates or oestradiol which inhibits activity brings about these *in vivo* changes by altering its synthesis or by some more direct mechanism has yet to be established.

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Behavioural Effects of some Derivatives of Amphetamine and LSD and their Significance

In attempts to work out possible mechanisms of action of the hallucinogenic drugs, it would be helpful to know which chemical groups modify their activity. In the methoxylated amphetamines, some methoxy groups may act as electrostatic binding sites and some may effect the shape and energy distribution of the π cloud. It may also be important to know whether the amino group is necessary for activity. It has been shown that N,N-dimethylmescaline is a less potent compound than mescaline[1,2]. We have now tested further N,N-dimethyl derivatives of hallucinogenic amphetamines using the Bovet-Gatti profiles on the Sidman avoidance schedule[3,4] which we have found to discriminate between hallucinogenic drugs very well. 4-Methoxyamphetamine is a very potent disrupting drug in rats and produces a typical "hallucinogenic" profile at 3 mg/kg (equivalent to mescaline at 25 mg/kg). The N,N-dimethyl derivative was totally inactive at 25 mg/kg. 2,5-Dimethoxy-4-methyl amphetamine (DOM) is the most potent human hallucinogenic amphetamine known (active in rats at 2·5 mg/kg). Its N,N-dimethyl derivative was also quite inactive at 20 mg/kg. These results suggest that, if these compounds bond, they do so using their N atoms, in which case our hypothesis[5] would predict that the 3-compound (not yet tested) would be an active hallucinogen. The relative activity of methoxylated amphetamines seems to correlate more with their quantum chemical properties, such as their Hückel molecular orbital (HMO) energies and degree of native fluorescence[6], than with their steric properties. That is to say, the steric requirements seem to be an unhindered N and an unhindered *ortho* or *para* methoxy group, and the energetic requirement is a high HMO energy.

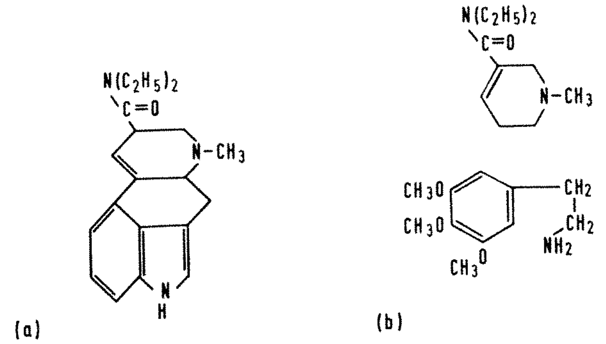

Fig. 1. (*a*) LSD; (*b*) mescaline and 1-methyl-1,2,5,6-tetrahydropyridine-3-(N,N-diethylcarboxamide).

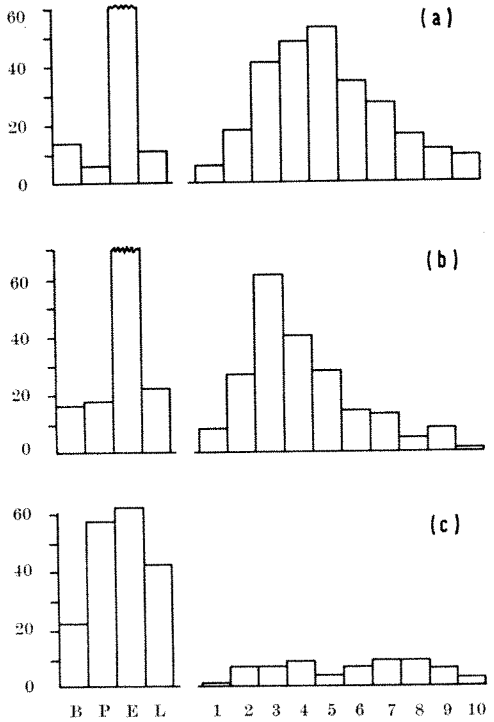

Fig. 2. The Bovet-Gatti profiles of the interaction between THPC and mescaline; (*a*) 15 mg/kg of THPC; (*b*) 12·5 mg/kg of mescaline; (*c*) the same, after treatment with 15 mg/kg of THPC. The latter is similar to the effect normally produced by 25 mg/kg of mescaline. The total number of responses in each category is given. B, Burst responses; P, premature responses; E, efficient responses; L, late responses; on the right the distribution of responses within the 10 s conditioned stimulus (CS) period is given. Hallucinogens increase B, P and L responses and shift the peak of the responses in the CS period to the left. The total number of E responses may be computed as the sum of the columns in the right-hand figure.

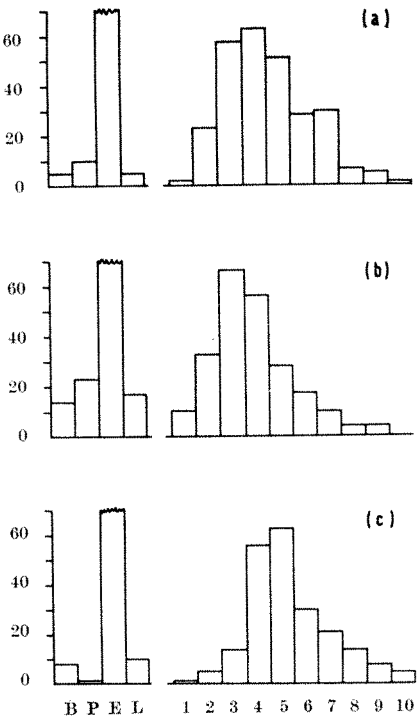

Fig. 3. The same for LSD: (*a*) saline; (*b*) 0·05 mg/kg of LSD; (*c*) 0·1 mg/kg of LSD after 15 mg/kg of THPC.

A second prediction of our hypothesis[5] is that blocking all three active groups on 5-HT (5-hydroxytryptamine) should inactivate the compound. 5-Methoxy-N,N-dimethyltryptamine is a most potent hallucinogen and disrupts rat behaviour at 2·5 mg/kg. We have tested the 1-methyl derivative of this drug and found it to have only a mild "stimulant" (amphetamine-like) profile at 10 mg/kg. This is in contrast to 1-methyl LSD which is an active hallucinogen.

We wished to know whether halving the LSD molecule and giving the bits separately would be effective. An approximation to this procedure can be carried out by determining whether 1-methyl-1,2,5,6-tetrahydropyridine-N,N-diethylcarboxamide (THPC; Fig. 1) potentiates the effect of mescaline. Fig. 2 shows that pre-treatment with 15 mg/kg of THPC, 45 min before treating with 12·5 mg/kg of mescaline, markedly potentiates the effects of the latter. This was confirmed in a second rat, the behaviour of which was completely disrupted for 30 min by the combination. When given before an equivalent dose of LSD (0·1 mg/kg), there was no such potentiation (Fig. 3), but the effect of LSD was inhibited. This dose of THPC by itself produced no behavioural effect (Figs. 2 and 3).

THPC could potentiate the effect of mescaline by one of two mechanisms: (i) if mescaline occupies only half the LSD site, as Snyder and Richelson have proposed[6], occupation of the other half by THPC might increase its effect, at the same time blocking binding of LSD, or (ii) THPC might affect some other brain mechanism, which, although it might have no direct behavioural effect, would nevertheless potentiate the effect of mescaline on some other system. But because mescaline and LSD are believed to act on the same mechanism, it is difficult to see why such a non-specific action should not potentiate the effect of LSD as well.

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Correlation between Activity and Electronic State of Hallucinogenic Amphetamines

THE actions of hallucinogenic agents have been correlated with Hückel molecular orbital calculations of the π-electrons[1] and with fluorescent properties[2]. The Hückel correlations on three methoxylated amphetamines indicated that hallucinogenic activity correlated with energy of the highest occupied molecular orbital[1].

We calculated the total valence electron charge density, frontier electron density, dipole moment, total energy and all energy levels of thirteen hallucinogenic amphetamines using the INDO method (intermediate neglect of differential overlap)[3] which gives good results in the calculation of dipole moments, molecular geometries, electron nuclear hyperfine coupling constants and force constants[4-7]. Standard bond lengths and bond angles were used to generate cartesian coordinates, and vicinal methoxy groups were regarded as out of plane in a *trans*-configuration. All results were subjected to sequential multiple regression analysis.

Table 1 shows the correlation between hallucinogenic activities in man[8] and the energy of the highest occupied molecular orbital (E_H). For this correlation, the linear regression equation, $\log A = a + bx_1 + cx_2 \ldots$, may be expressed by the following equation, where A is hallucinogenic activity

$$\log A = 19{\cdot}07 + 35{\cdot}65\ E_H \qquad (1)$$

The equation for equimolar activity is

$$\log A = 18{\cdot}7 + 35{\cdot}1\ E_H \qquad (2)$$

For equation (1), $r = 0{\cdot}753$, $F = 14{\cdot}36$ and $P < 0{\cdot}005$; for equation (2), $r = 0{\cdot}756$, $F = 14{\cdot}62$ and $P < 0{\cdot}005$. This correlation suggests that the ease of perturbability of the π-electrons of the amphetamines is a determinant of hallucinogenic activity, the amphetamines presumably forming with the brain receptor a low energy, reversible π-molecular complex that gives rise to hallucinations.

Table 1. HALLUCINOGENIC ACTIVITIES OF AMPHETAMINE DERIVATIVES IN MAN AND ENERGY OF THE HIGHEST OCCUPIED MOLECULAR ORBITAL (E_H)

Derivative of amphetamine	Hallucinogenic activity	E_H (atomic units)
2,5-$(OCH_3)_2$-4-CH_3	80–100*	−0·4929
2,4,5-$(OCH_3)_3$	17	−0·5001
2,3,6-$(OCH_3)_3$	13	−0·5112
2,4,6-$(OCH_3)_3$	10	−0·5217
2,5-$(OCH_3)_2$	8	−0·5012
2,3,4,5-$(OCH_3)_4$	6	−0·5126
2,4-$(OCH_3)_2$	5	−0·5194
4-OCH_3	5	−0·5262
2,3,5-$(OCH_3)_3$ †	4	−0·5026
3,4,5-$(OCH_3)_3$	2	−0·5218
2,3,4-$(OCH_3)_3$	< 2	−0·5274
3,4-$(OCH_3)_2$	< 1	−0·5238
(Mescaline) ‡	1	−0·5226
(Amphetamine)	0	−0·5885

* The activity has been reported as 80 and 100[2,8].

† Limited number of tests.

‡ Mescaline, 3,4,5-trimethoxypherylethylamine, is not an amphetamine derivative but is used as a standard of hallucinogenic activity[8]. Amphetamine is not hallucinogenic and is not considered in the statistical analysis.

Table 2. E_H OF SOME UNTESTED AMPHETAMINE DERIVATIVES AND THEIR PREDICTED HALLUCINOGENIC ACTIVITIES

Derivative of amphetamine	E_H (atomic units)	Predicted activity
2,3,4,6-$(OCH_3)_4$	−0·5067	10·0
3,5-$(OCH_3)_2$	−0·5240	2·4
4-OH	−0·5296	1·5
3-OCH_3	−0·5316	1·3
2,6-$(OCH_3)_2$	−0·5334	1·1
2,3-$(OCH_3)_2$	−0·5346	1·0
2-OCH_3	−0·5371	0·9

Equation (1) was used for the predictions; equation (2) gives very similar results.

Although the correlation is significant ($r = 0{\cdot}753$, $F = 14{\cdot}36$, $P < 0{\cdot}005$), E_H does not account for the activities of all the compounds. The 2,3,6-trimethoxy and 2,4,6-trimethoxy derivatives and the 4-methoxy derivative are more active than expected if E_H is the only determinant of activity; and the 2,3,5-trimethoxy is less active. Other electronic or steric characteristics not revealed by this work could contribute to the activity. A better correlation cannot be expected. Drugs are involved in numerous interactions[9] before they induce hallucinations. Also, the error in measuring relative hallucinogenic activity in man is about 20 per cent (personal communication from A. T. Shulgin). Assuming that E_H is the only correlate of activity, we calculated the expected hallucinogenic activity of some untested amphetamines (Table 2). Of these, the 2,3,4,6-tetramethoxy derivative is the only one that should be significantly more potent than amphetamine. Shulgin states in a personal communication that the 2,3,4-trimethoxy derivative has measureable activity, but it is less than two[8]; our calculations predict that it has about the same activity as mescaline.

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Rat Embryo Cells in Complement Fixation Tests for Murine Leukaemia Virus

HARTLEY *et al.*[1] have described a sensitive technique for the detection and quantitation of murine leukaemia viruses based on the development of complement fixing group specific antigen in infected mouse embryo (ME) tissue culture. This depends on the use of rat antisera in the complement fixation test to detect antigens shared by all known murine leukaemia viruses[2,3]. A number of tissue culture cells were tested for their ability to develop complement fixation antigen, and it was found that Swiss NIH-ME cells were the most sensitive host cells[1]. We have recently established and studied continuous lines of rat embryo (RE) cells—uninoculated and infected with Rauscher leukaemia virus (unpublished data of J. S. R. *et al.*)—and found that RE cells, as well as ME cells, can be used for the cultivation of Rauscher leukaemia virus. We now describe the use of RE cells in the test of Hartley *et al.*

The murine leukaemia and sarcoma virus strains used for this study had been serially passaged by Dr J. W. Hartley in NIH-ME cells and we passaged them several more times in the same type of culture before commencing our studies. Primary NIH-ME and Fisher RE cells were obtained from Microbiological Associates, or were prepared as previously described[1]. The growth medium was Eagle's minimal essential medium with 2 mM glutamine, 100 U penicillin and 100 µg streptomycin per ml.; and 10 per cent unheated foetal bovine serum; this was also used to maintain the cultures used for production of leukaemia virus, and for complement fixation antigen pools. Murine leukaemia viruses were assayed in secondary NIH-ME cultures by Hartley's test[1]. Complement fixation tests were carried out by the microtitre technique[4]. Titres were recorded as the reciprocal of the highest dilution giving 3+ to 4+ fixation of 1·8 units of complement. Rat antisera used in the complement fixation test were obtained from Fisher rats carrying transplanted sarcomas induced by Moloney sarcoma virus.

In the first experiment, 16 fluid ounce bottle cultures of secondary NIH-ME, RE and the eleventh passage of RE cells, were infected with 10^4 infectious units of Rauscher leukaemia virus; these cultures were harvested on the fourteenth day after inoculation. The media were removed and saved for infectivity assay, and the cells were packed for complement fixation antigens as previously described[5]. Table 1 indicates the ability of NIH-ME and RE cells and a continuous, normal cell line of RE cells (eleventh passage) to produce complement fixation antigen and yields of virus following inoculation with Rauscher leukaemia virus. A complement fixation titre of 1 : 32 was attained in NIH-ME and RE cells, while 1 : 8 was produced in the continuous RE cell line. Virus yields obtained in RE cells and the continuous RE cell cultures were higher than those in ME cells.

Table 1. PRODUCTION OF COMPLEMENT FIXING ANTIGENS AND YIELDS OF RAUSCHER LEUKAEMIA VIRUS IN RE (FISHER) AND ME (NIH SWISS) CELLS 14 DAYS AFTER INOCULATION

Cells	Antigen titre* against sarcoma virus : rat serum pool 6	Virus† yields
RE (secondary)	1 : 32	4·4
Serial RE (twelfth passage)	(8)	4·4
ME (secondary)	1 : 32	3·4

* Complement fixation titre = reciprocal of dilution giving 3+ to 4+ fixation of 1·8 units of complement (in parentheses indicates 2+).

† Complement fixing antigen induction titre ($\log_{10}$ TCD_{50}/ml.).

In the second experiment the sensitivity of NIH-ME and RE cells for the assay of Rauscher leukaemia virus was examined. The same stock of Rauscher leukaemia virus was titrated at the same time by the complement fixation antigen induction test[1], using two 60 mm plastic Petri dishes of secondary NIH-ME and RE cells for each ten-fold dilution. The results (Table 2) demonstrate that the endpoint titres of Rauscher leukaemia virus were the same in ME and RE cells.

Table 2. COMPARATIVE TITRATION OF RAUSCHER LEUKAEMIA VIRUS IN RE (FISHER) AND ME (NIH SWISS) CELLS 20 DAYS AFTER INOCULATION

Cells	Virus dilution	Sarcoma virus : rat serum pool 6 (1 : 4)	Infectivity titre (Log_{10} TCD_{50}/0·4 ml.)
RE	10^{-1}	4	$10^{-3\cdot0}$
	10^{-2}	4	
	10^{-3}	4	
	10^{-4}	0	
	10^{-5}	0	
ME	10^{-1}	4	$10^{-3\cdot0}$
	10^{-2}	4	
	10^{-3}	3	
	10^{-4}	0	
	10^{-5}	0	

Additional experiments were designed to examine the comparative sensitivity of rat strains for complement fixation antigen production of Rauscher leukaemia virus. Different strains of rat were obtained from Microbiological Associates, and primary cultures of rat embryo were prepared as described. Table 3 shows the results of comparative sensitivity of rat strains for complement fixation antigen production of Rauscher leukaemia virus. All the RE cells derived from different strains yielded comparable complement fixation antigens with NIH-ME cells, the highest titre being that for the "OM" rat strain. No positive complement fixation reactions were found in cell pack preparations of the normal, uninoculated RE cells.

Table 3. COMPARATIVE SENSITIVITY OF RAT STRAINS FOR COMPLEMENT FIXING ANTIGEN PRODUCTION OF RAUSCHER LEUKAEMIA VIRUS (RLV), INOCULATED (+) OR UNINOCULATED (−)

Cell	Strain	RLV	Titres* against sarcoma virus rat serum 8
RE	Fisher-344	+	1 : 16
		−	0
	ACI	+	1 : 32
		−	0
	BUF	+	1 : 8
		−	0
	LEW	+	1 : 8
		−	0
	WF	+	(16)
		−	0
	Wistar	+	1 : 8
		−	0
	OM	+	(64)
		−	0
ME	NIH Swiss	+	(32)
		+	(16)
		−	0

* See Table 1; 0 = 0/4.

NIH-ME and RE cells were further tested for comparative sensitivity in their ability to produce complement fixation antigen of various established murine leukaemia and sarcoma virus strains. Various stock viruses were inoculated into ME and RE cells at the same time, and harvested on the same day. Complement fixation antigen titres in RE cells were in almost all instances as high as, or higher than, those obtained in NIH-ME cells (Table 4).

Our results indicate that RE cells seem to be useful for the propagation and assay of murine leukaemia and sarcoma viruses cultured *in vitro*. Complement fixing antigen titres and yields of virus produced in rat embryo cells are similar to those obtained in NIH-ME cells, the most sensitive host cell system so far reported[1]. RE cells provide an additional system for use in Hartley's test, and may have advantages for the isolation of certain viruses. We hope to study the comparative sensitivity of RE cells for virus yields of various murine leukaemia viruses and for the isolation of viruses from field specimens of leukaemia. Ting[6] observed that Moloney sarcoma virus induced morphological transformation of rat embryo cells *in vitro*, and infectious viruses were found in the culture media of the transformed culture. Continuous viral replication and malignant cell transformation by

Table 4. PRODUCTION OF COMPLEMENT FIXING ANTIGENS OF MURINE LEUKAEMIA AND SARCOMA VIRUSES IN RE (FISHER) AND ME (NIH SWISS) CELLS

Murine leukaemia sarcoma viruses*	Days after inoculation	Titres† against sarcoma virus rat serum RE cells	ME cells
Leukaemia viruses			
Moloney	18	8	16
Rauscher	18	> 16	> 16
Friend	18	> 16	> 16
High leukaemic strains			
AKR	18	> 16	> 16
AKR-E	20	32	8
C58	16	8	8
C3H/FgE 3	16	4	4
Low leukaemic strains			
C3H/Hen-E 2	20	8	16
CF-1	20	> 8	8
BALB/c N	20	8	8
BALB/c T	30	> 8	< 4
Sarcoma viruses			
Moloney sarcoma	18	> 16	16
Harvey sarcoma	18	> 8	> 8
Finkel osteosarcoma	20	4	> 4

* The tissue culture-grown murine leukaemia and sarcoma virus strains were furnished by Dr J. W. Hartley[1,3].

† Complement fixation titres = reciprocal of dilution giving 3+ to 4+ fixation of 1·8 units of complement.

Finkel's osteosarcoma virus have recently been reported in rat embryo cells[7].

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Serological Studies with EB Virus in Infectious Lymphocytosis

RECENT epidemiological studies reveal a highly significant correlation between infectious mononucleosis and the development of serum antibodies to EB virus (EBV)[1,2]. It has been suggested that these findings indicate that EBV is probably the causative agent in infectious mononucleosis. An alternative hypothesis is that EBV may merely be a poorly immunogenic, non-aetiological "passenger" virus that is activated non-specifically during the proliferation of lymphoreticular cells in infectious mononucleosis with subsequent appearance of detectable EBV antibodies[3–8]. The second hypothesis may be tested in part by studying EBV antibody responses that occur in infectious lymphocytosis, a mild communicable paediatric illness of unknown aetiology in which elevated absolute and relative lymphocyte counts develop, usually without specific signs or symptoms[9].

An outbreak of infectious lymphocytosis occurred in 1960 at Junior Village, the receiving home for the Washington, DC, Department of Welfare. (This community consists of homeless but otherwise normal children.) At the time of this outbreak, the infectious disease experience of Junior Village children was under continuous longitudinal surveillance[10] and serial serum specimens were routinely collected from all children and stored at −20° C. During the summer and early autumn of 1960 infectious lymphocytosis was detected in twenty-two children.

Acute and convalescent sera from ten of the children were studied for antibodies to EBV as well as to cytomegalovirus (CMV) and herpes simplex virus (HSV), all herpes group viruses. CMV has been implicated in mononucleosis syndromes[11] and antibody to CMV was therefore studied. HSV testing was included as a control herpes group virus. The indirect immunofluorescence test for detection of EBV antibody was used[12] using the Jiyoye Burkitt tumour cell line as antigen. Controls were included to rule out staining by immunoglobulin G producing cells. The complement fixation test for CMV and HSV was used as described elsewhere[13]. None of the children developed a four-fold or greater antibody rise to EBV or CMV, although the lymphocytosis was impressive in each instance (Table 1).

Table 1. SEROLOGICAL STUDIES IN CHILDREN WITH INFECTIOUS LYMPHOCYTOSIS

Child	Peak white blood cell count*		Date of serum	EBV antibody titre	CMV antibody titre §	HSV antibody titre
L.C.	29,800	(96%)	Sep. 8 1960	< 10†	< 10	40
			Oct. 27 1960	< 10	< 10	20
D.E.	63,000	(84%)	Jun. 17 1960	80	< 10	20
			Sep. 27 1960	80	< 10	20
B.G.	51,200	(82%)	Jun. 20 1960	< 10	AC‡	AC
			Sep. 22 1960	< 10	AC	AC
C.G.	55,707	(80%)	Jun. 17 1960	< 10	< 10	20
			Sep. 22 1960	< 10	< 10	20
T.H.	73,600	(91%)	Jun. 17 1960	< 10	< 10	20
			Aug. 31 1960	< 10	< 10	40
			Sep. 22 1960	< 10	< 10	40
M.J.	47,000	(81%)	Jun. 20 1960	< 10	< 10	20
			Sep. 22 1960	< 10	< 10	40
J.P.	70,800	(85%)	Jun. 17 1960	80	< 10	10
			Sep. 22 1960	80	< 10	< 10
L.P.	73,600	(87%)	Jun. 14 1960	< 10	< 10	< 10
			Oct. 27 1960	< 10	< 10	< 10
R.P.	81,000	(90%)	Jun. 20 1960	40	< 10	20
			Sep. 20 1960	80	< 10	80
			Jan. 3 1961	80	< 10	20
			Apr. 18 1961	80	< 10	40
W.P.	50,300	(83%)	Jun. 20 1960	80	< 10	< 10
			Sep. 22 1960	80	< 10	< 10

* Numbers in parentheses indicate percentage lymphocytes.

† Titres expressed as reciprocal of serum dilution.

‡ Serum anti-complementary.

§ Positive control serum included in test.

Thus, according to serological testing, EBV and CMV are not associated with the pathogenesis of infectious lymphocytosis. Further, the failure to find an EBV antibody rise in any of the children indicates that EBV, if present in trace amounts in three children, did not become activated during the striking proliferation of their lymphocytes. This may have implications in the interpretation of the role of EBV in infectious mononucleosis.

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Programmed Synthesis and Export of Immunoglobulin by Synchronized Myeloma Cells

THE mammalian cell life cycle is determined by a series of coordinated events which reflect time-dependent gene expression, although the duration of some of these events appears to be flexible[1]. Examples of both cyclic[2] and steady state[3] activities of certain enzymes in different, synchronized mammalian cell lines have been reported; there is at least one known example[3] of enzyme inducibility that is restricted to part of the cell cycle. Collagen, a protein synthesized in large amounts for export, is produced throughout the life cycles of several fibroblast lines at rates corresponding to those of overall protein synthesis[4].

The chronology of immunoglobulin production by cultured lymphoid cells is of special interest. Immunoglobulins are readily identifiable proteins secreted in large quantities, and control of their synthesis is of central importance in the immune response. Although there is the possibility that regulation of immunoglobulin synthesis in myelomas may be different from that in normal cells, cultured myeloma cells provide a useful model for analysing the regulation of immunoglobulin synthesis, particularly because they produce a homogeneous protein.

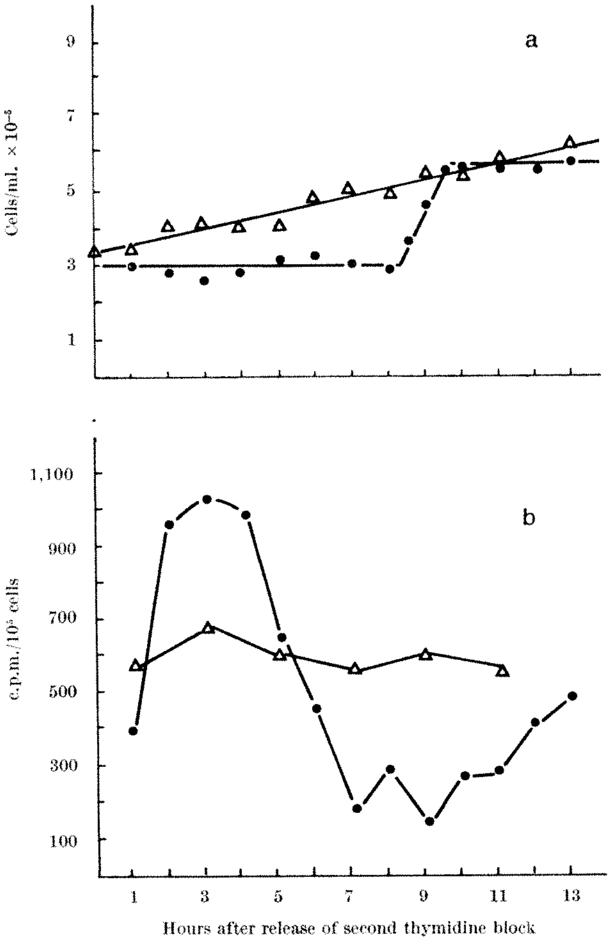

Fig. 1. *a*, Cell counts in synchronized and asynchronous cultures of C1 myeloma cells. Cell numbers were counted at hourly intervals after the removal of the second thymidine block. ●, Thymidine treated cells; △, randomly growing control cells. *b*, Thymidine uptake into DNA of synchronized and asynchronous cultures. ^{3}H-thymidine (0·04 μCi/ml.) was added at hourly intervals, for 1 h pulses, to a series of 5 ml. aliquots of C1 myeloma cells after the release of the second thymidine block. At the end of the pulse time, cells were collected on 'Millipore' filters, washed with Hanks solution and then DNA was precipitated with 5 per cent trichloroacetic acid. The filters were dried and counted in a liquid scintillation spectrometer using toluene phosphor. Control asynchronous cell cultures were treated as above, but were pulsed for 1 h periods every second hour. ●, Thymidine treated cells; △, randomly growing control cells.

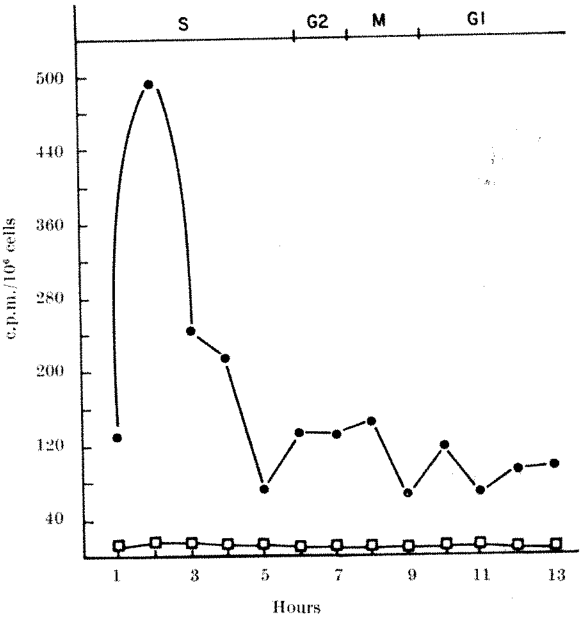

Fig. 2. Pulse-labelling of γG_{2a} in synchronized cultures of C1 cells. Beginning immediately after the release of the second thymidine block, a series of 10 ml. aliquots of cells were pulse-labelled for 1 h, at hourly intervals, with a mixture of ^{3}H or ^{14}C-labelled amino-acids (0·5 μCi/ml. each of glutamic acid, aspartic acid, proline, valine and glycine). Soluble cell extracts were prepared as follows. Cells were harvested by centrifugation and culture medium was removed. The pellet was washed three times with Hanks solution, lyophilized, then dissolved in water. The suspension was centrifuged at 100,000*g* for 60 min, the supernate removed, lyophilized and then taken up in a known volume of 0·05 M barbital, *p*H 8·6. The culture medium was dialysed against distilled water for 48 h and centrifuged at 12,000*g* for 10 min to remove insoluble euglobulins. The supernatant was removed and $(NH_4)_2SO_4$ added to 45 per cent saturation. The precipitate was collected by centrifugation, dissolved in water, dialysed against distilled water, lyophilized, then taken up in a known volume of barbital buffer. All samples were assayed for labelled γG_{2a} by co-precipitation[8] with unlabelled γG_{2a} myeloma protein, using goat anti-γG_{2a} antiserum (kindly provided by Dr B. W. Papermaster). Precipitates were collected on 'Millipore' filters and counted as described in the legend to Fig. 1. In this and following figures, the time of labelling is given in hours after release of the second thymidine block (which was designated as the beginning of S phase). ●, Cell extracts; □, culture media.

Many myeloma cell lines are now available in continuous culture[5]. One such line, C1, derived from the C3H mouse plasmacytoma X5563, synthesizes a γG_{2a} immunoglobulin[6]; a derived negative mutant, XC1, is also available. These two cell lines have different chromosome numbers[7] and the C1 cell line carries a virus-like particle not visible in XC1 (ref. 5). We have examined the synthesis and secretion of γG_{2a} immunoglobulin by C1 cells synchronized in suspension culture by double thymidine block. Both synthesis and export of γG_{2a} are time-ordered events, reaching maximal rates during restricted portions of the cell life cycle.

C1 and XC1 cell lines were established in continuous suspension culture in a modified Eagle's medium from initial cultures provided by Drs M. Cohn and K. Horibata (Salk Institute, San Diego). The most effective conditions for establishing synchronous growth were as follows: cells were subcultured in fresh medium at 2×10^5 cells/ml., grown for 16 h with 10^{-3} M thymidine, 7 h in the absence of thymidine and a further 16 h with 10^{-3} M thymidine. After removal of the second thymidine block, the extent of synchrony was assessed by serial cell counts and measurement of ^{3}H-thymidine incorporation into DNA (Fig. 1). Control asynchronous cells had a doubling time of 16 to 18 h. In thymidine treated cultures, S phase occupied 6–7 h, G2 phase 1·5–2 h, M phase 1·5–2 h and G1 phase about 3–4 h, giving a total generation time slightly less than that of asynchronous cultures. Approximately 90 per cent synchrony was obtained by this procedure.

In radiolabelling experiments, synchronized cultures were incubated with radioactive amino-acids for the required time and radiolabelled γG_{2a} was assayed in soluble cell extracts and in the medium by co-precipitation[8]. In pulse-labelling experiments, radioactive amino-acid incorporation into γG_{2a} was measurable at all times during the cell life cycle but was clearly maximal early in S phase (Fig. 2), reflecting time-dependent regulation of γG_{2a} biosynthesis. The basal level of synthesis was rather higher than could be accounted for by a random distribution in time of S phase-limited synthesis of γG_{2a} in the small proportion of asynchronous cells present. Little or no labelled γG_{2a} appeared in the medium in a 1 h pulse time (Fig. 2), so that the amount of radiolabel appearing in intracellular γG_{2a} during 1 h does reflect net synthesis of immunoglobulin in this period.

In continuous labelling experiments, in which radioactive amino-acids were added following removal of the second thymidine block and hourly samples assayed thereafter, the rate of incorporation of label into cellular γG_{2a} was non-linear, being maximal early in S phase (Fig. 3*a*). Label was not limiting, for the incorporation into total cell protein was linear for at least 9 h. A similar initial slope for incorporation of isotope into γG_{2a} was obtained by plotting cumulative counts of samples sequentially pulse-labelled in S phase. The rate of appearance of labelled γG_{2a} in the medium was also non-linear, with an initial lag followed by an increasing rate of export of newly synthesized immunoglobulin which reached a maximum in G2 and M phases (Fig. 3*b*). There was thus a dissociation in time between maximal synthesis and maximal secretion of γG_{2a}, the intervening lag period being of the order of hours rather than minutes.

Total γG_{2a} (labelled and unlabelled) was determined by immunodiffusion assay. Intracellular γG_{2a} rose to an apparent steady state concentration in mid S phase, which was maintained until G2 and then decreased (Fig. 3*c*). The rate of accumulation of γG_{2a} in the medium increased after an initial lag (Fig. 3*d*). In other experiments, the accumulation of intracellular γG_{2a} was also calculated from radiolabelling data, by plotting labelled γG_{2a} as a percentage of total (soluble and insoluble) labelled cellular protein measured at hourly intervals. Here the intracellular concentration of newly synthesized γG_{2a} was non-linear and reached a maximum in late S phase, intermediate in time between the periods of maximal synthesis and maximal export. In contrast to the cyclic synthesis of γG_{2a}, the synthesis of total cellular protein was approximately linear until mitosis. Intracellular labelled γG_{2a} reached a maximum of 1·5–2 per cent of total labelled cellular protein in synchronized cells.

We conclude, first, that both biosynthesis and export of γG_{2a} immunoglobulin by C1 myeloma cells have cyclic maxima, which occur during different, restricted portions of the cell life cycle and are separated by an intervening period of intracellular accumulation (Fig. 4). These findings indicate that both synthesis and export follow a programme determined in some way by the timing of other events in the cell life cycle. Second, the non-steady state synthesis of immunoglobulin contrasts with the above-mentioned pattern of collagen synthesis in fibroblasts[4]. Despite the species difference, it is of interest

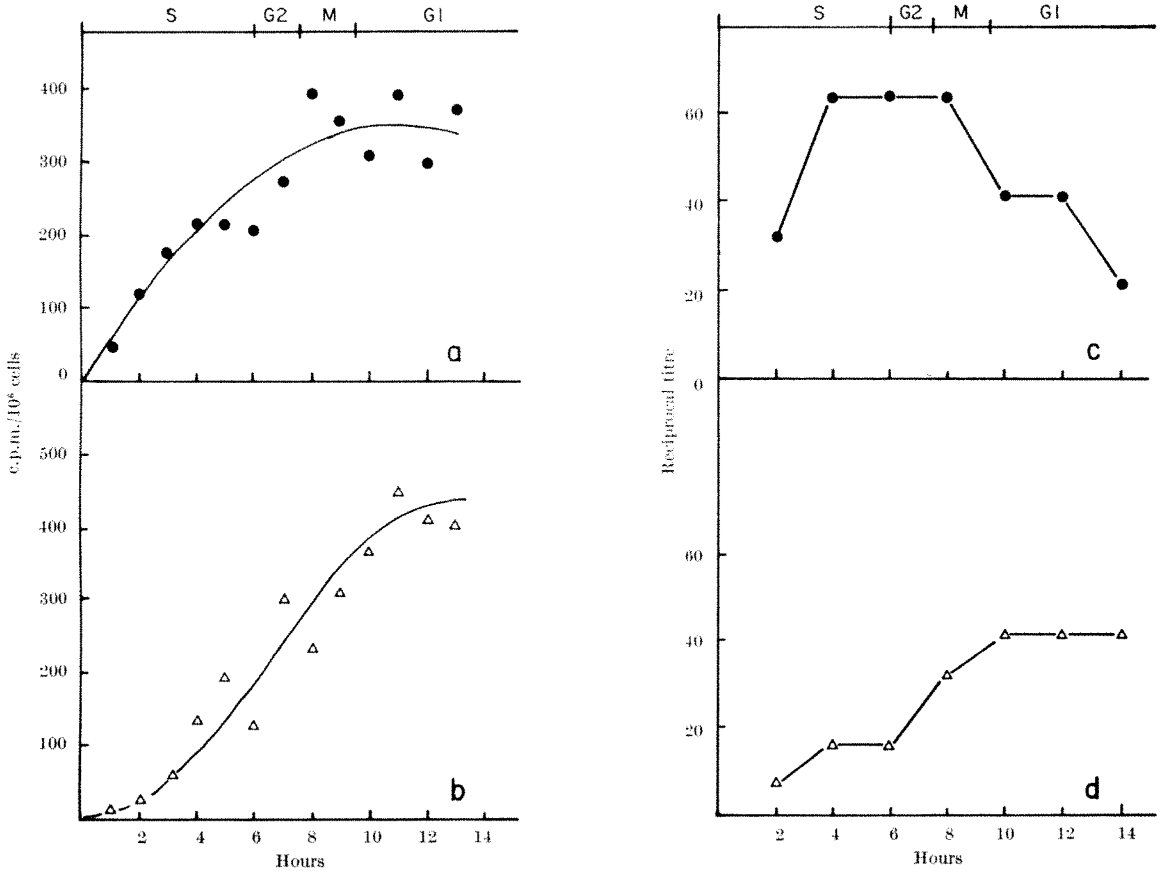

Fig. 3. *a* and *b*, Continuous labelling of γG_{2a} in synchronized cultures of C1 cells. Radioactive amino-acids (0·2 μCi/ml. each of glutamic acid, aspartic acid, proline, valine and glycine) were added to the cultures immediately after removal of the second thymidine block and samples taken at hourly intervals thereafter. Cell extracts and medium were prepared and assayed as described in the legend to Fig. 2. ●, Cell extracts; △, culture media. *c* and *d*, Determination of total γG_{2a} in cell extracts and culture media of synchronized cultures of C1 myeloma cells by immunodiffusion. Cell extracts and media were processed as described in the legend to Fig. 2. Serial, doubling dilutions of each sample were made; 5 μl. of each placed in the outer wells of Ouchterlony slides and 5 μl. of anti-γG_{2a} (diluted 1/5) placed in the centre well. Diffusion was carried out at 4° C for 48 h; the slides were then washed in barbital buffer for 24 h and stained with thiazine red. The γG_{2a} titre was taken as the greatest dilution giving a clearly visible band. The discrepancy between γG_{2a} content of cells and media in the 12–14 h period is evidently because of the limitations of the technique. ●, Cell extracts; △, culture media.

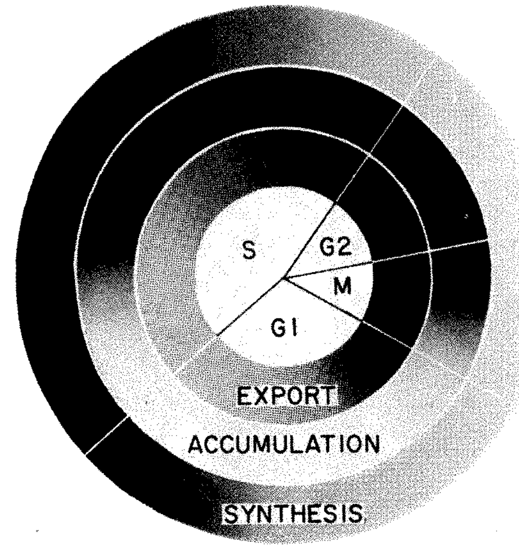

Fig. 4. Schematic representation of synthesis, intracellular accumulation and export of γG_{2a} as a function of time during the life cycle of C1 myeloma cells. The γG_{2a} concentration is proportional to the density of the shading within each zone.

to compare the present data for a mouse myeloma with those reported recently for two human lymphoid cell lines[9]. In the latter case, maximal synthesis, as judged by immunofluorescent staining, was observed in late G1 and early S phases; this was followed closely, however, by maximal export in contrast to the situation in the myeloma. Third, it can be inferred that in C1 myeloma cells—and apparently in the human lymphoid cells—immunoglobulin biosynthesis is inducible, possibly by an intrinsically generated inducer. Alternatively, by analogy with tyrosine aminotransferase induction in synchronized hepatoma cells[3], periodically permitted operation of an extrinsic inducer present in the medium could be responsible. The negative mutant, XC1, is attractive from the viewpoint of further examination of the induction process, but so far we have failed to induce γG_{2a} synthesis in this mutant (unpublished data).

Two questions can now be posed. First, does the periodicity of γG_{2a} synthesis reflect a restricted period of transcription of the corresponding messenger RNAs late in G1 phase ? If so, the kinetics of γG_{2a} synthesis might simply reflect the survival–decay curves of these messenger RNAs. Second, what governs the kinetics of export ? On the basis of current knowledge, the most likely factor is addition of the carbohydrate moiety of the immunoglobulin[10,11]. Possibly one or more relevant enzymes are also subject to cyclic induction in relation to the phase of cell life cycle. These two possibilities can be tested experimentally.

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Histones and Gene Control

HISTONES are thought to control the transcription of DNA in some way. There are five main fractions in the nuclei of mammalian, bird, fish and plant somatic cells: F1 and F2B, lysine-rich histones; F2A1 and F3, arginine-rich histones; and F2A2, an intermediate type[1].

Allfrey, Littau and Mirsky[2] and Hindley[3] have concluded that the arginine-rich histones are the best repressors of DNA template activity, whereas Barr and Butler[4] and Huang, Bonner and Murray[5] reported that the lysine-rich histones were the most efficient. We now show how these differences can be reconciled, and question the currently accepted view that the histones must be removed from the DNA before transcription can occur.

Histone fractions vary considerably in their ability to precipitate a given quantity of DNA[6]. F1, the lysine-rich histone, will precipitate all the DNA at a ratio of histone to DNA of about 0·8 to 1·0, whereas F3 requires a ratio of about 1·5 to 1·0 for complete precipitation. More recent work[7] has shown that in the correct conditions further additions of histone cause the precipitated DNA–histone complex to redissolve. Does the DNA–histone complex become available again as a template for RNA synthesis, as it redissolves, even though it contains more histone ? Fig. 1 shows that it does.

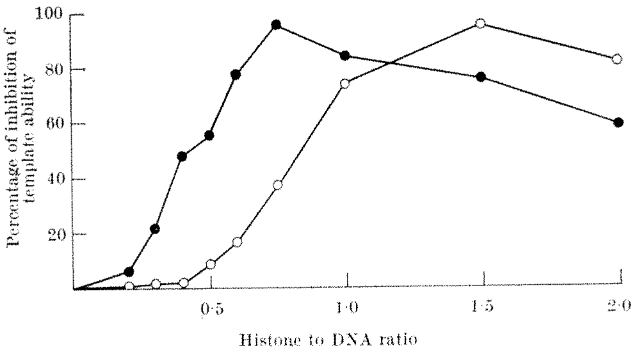

Fig. 1. The inhibition by histone fractions of the ability of DNA to act as a template for RNA synthesis *in vitro*. ●, Lysine-rich histone F1; ○, arginine-rich histone F3. Histones F1 and F3 were prepared according to Johns[8], method 2. DNA was prepared by the method of Kay, Simmons and Dounce[9]. RNA polymerase was prepared by a modification of the procedure of Chamberlin and Berg[10] and used at the stage designated fraction 3. To assay for RNA synthesis at the various histone to DNA ratios, varying amounts of the F1 and F3 fractions in a constant volume (50 μl.) of 0·14 M NaCl were added to aliquots (50 μl.) of DNA solution (2 mg/ml.) in 0·14 M NaCl to give histone to DNA ratios varying from zero to 2 : 1. The assay reagent mixture was then added: 0·05 M Tris-HCl buffer *p*H 7·8 (0·5 ml.) containing 4 μmoles $MgCl_2$; 1 μmole $MnCl_2$; 1 μmole β mercaptoethanol; 200 nmoles each of ATP, CTP, GTP, UTP and 0·55 nmoles ^{14}C-ATP (specific activity, 89 mCi/mmole). The reaction was started by adding RNA polymerase (10 μl. containing 300 μg protein). After incubation at 37° C for 10 min the reaction was stopped by adding 3 ml. of 0·5 M perchloric acid and the precipitate was collected on Whatman 'GF/C' filters, washed with 15 ml. 0·5 M perchloric acid and 10 ml. ethanol and dried. The filters were counted in 10 ml. toluene-based phosphor in a Packard liquid scintillation counter. Controls were as shown in Table 1. The figures given are averages of five experiments. All assays were carried out in duplicate.

The ratios of histone to DNA for complete precipitation using F1 and F3 are 0·8 and 1·5 to 1·0 respectively. Fig. 1 shows that at these ratios there is virtually complete inhibition of RNA synthesis. This is not surprising, for it is unlikely that the precipitated DNA can act as a template. Further additions of histone cause the complex to become more soluble and to direct RNA synthesis once more. The discrepancies between the results of the various authors can be reconciled if the ratios of histone to DNA used in their experiments are taken into account. When DNA is precipitated with an equal amount of various histones, the ratio histone to DNA being 1·0 (Huang, Bonner and Murray[5] and Barr and Butler[4]), or at any ratio less than 1·0 (see Fig. 1), F1 is the best repressor. At the higher histone to DNA ratios (Hindley[3]), F3 is the best repressor. The ratio of histone to DNA used in the experi-

Table 1. INCORPORATION OF ^{14}C-AMP INTO PERCHLORIC ACID PRECIPITATE

	pmoles ^{14}C-AMP incorporated
Complete reaction mixture (no histone, 100 μg DNA)	7,800
Complete reaction mixture (zero time)	Zero
Complete reaction mixture + ribonuclease	200
Reaction mixture less enzyme	Zero
Reaction mixture less DNA	400

ments of Allfrey *et al.*[2] is difficult to assess because histone was added directly to nuclei. In these conditions the histones would be well in excess of the DNA and the arginine-rich histone F3 seems to be the best repressor.

We suggest that up to the point of complete precipitation of DNA by added histones the curves of inhibition of template activity (Fig. 1) and the curves of precipitation of DNA by histone[6] are so similar that the assay for template ability is simply a very sophisticated method for measuring DNA precipitation. The increase in template ability when the precipitate is redissolved by more histone raises a number of interesting questions.

Is the restriction of template ability by histones as measured *in vitro* simply a measure of the inability of the reagents to penetrate and react with the aggregated complex? Is it necessary to remove the histones from the DNA–histone complex to increase the template ability of the DNA, or is it only necessary to render the whole complex more soluble or more accessible to reagents? Is it possible that *in vivo* RNA synthesis is controlled in this manner?

There is no evidence at present which indicates that the histones must be removed from the DNA before it can be used as a template for the *in vivo* synthesis of RNA. There is good evidence, however, that in the active regions of Drosophila salivary glands[11], and in the active diffuse chromatin of interphase lymphocytes[12], there is an increase in the acidic protein content of the region; but a decrease in the content of histone relative to the DNA has never been demonstrated.

It is possible that specific acidic proteins[13] combine with the required portion of the genome to render it more accessible to the RNA synthesizing system. A possible mechanism for this, involving the steric hindrance of the RNA polymerase by the DNA supercoil, has been discussed recently[14].

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Changes in the Amount of DNA in Cell Nuclei during Vertebrate Evolution

THE pronounced differences in the DNA content of cell nuclei of vertebrates have recently been discussed[1-3], but these articles did not mention the possible correlations between the evolution of nuclear DNA with the evolution of cell size which was simultaneously reviewed[4]. I now suggest that these two evolutionary phenomena are closely linked and result from common causes.

Many authors have observed a nearly perfect correlation between the size of the nucleus and the amount of DNA[5-9]. Others have denied the existence of such regularity or have insisted that there are exceptions[10-13], emphasizing that the nuclei are not only composed of DNA, and that the variations in the amount of remaining components as induced, for example, by starvation or by different stages of secretory cycle, cause changes in the nuclear size without affecting the amount of DNA. This being so, a perfect linear relationship between these two values cannot be expected. Nevertheless, it is obvious that the DNA is a very important component of the nucleus and that the differences in its amount are strongly reflected in the size of the nucleus. Fankhauser[14], for example, has demonstrated that the polyploid amphibian nuclei can invariably be recognized by their size.

The correlations between the nucleus and the cell are generally recognized[15-17]. Recently, Uzzell[18] used the size of the erythrocyte for the recognition of polyploids in the *Ambystoma jeffersonianum* complex. *Amphiuma*, which has the largest amount of DNA in its nuclei, also has the largest cells. The nucleocytoplasmic ratio may, of course, fluctuate[19], but it is true to say that when homologous cells in a similar functional state are compared the nucleocytoplasmic ratio is usually found to be uniform.

When the ratio of the nuclear volume to cell volume is calculated in cells of different size, it is usually found that in the larger cells the ratio is shifted toward the nucleus[20] (not toward the cytoplasm as I have erroneously stated[4]); that is to say, big cells have nuclei that are proportionally larger.

The next step of the reasoning, namely, the premise that the size of the cell has adaptive value and has been accordingly influenced by natural selection, is probably the most controversial and needs more verification from observations and experiment. In a recent article[4], I concluded that large cells probably have the metabolic rate limited by the surface to volume ratio. In those vertebrate groups which have increased their metabolism during evolution, the cell size has correspondingly decreased. Large cells are, however, more economic than small ones, so that in every instance when the metabolic level decreased, there was a tendency toward an increase in cell size. Godula[21] has compared the amount of some organelles in the liver cell of *Xenopus laevis* and *Ambystoma mexicanum* with that reported for the rat liver cells, and has found that, although the liver cell of *Xenopus* is about 40 per cent larger and that of *Ambystoma* about five times larger than the rat liver cell, the total number of mitochondria in one cell is 1,060 in the rat, 242 in *Xenopus* and 899 in *Ambystoma*. He also noted that the mitochondria in *Ambystoma* seem to have a smaller number of cristae than those of *Xenopus*. Goniakowska (in preparation) has found that the largest erythrocytes investigated (of axolotl) consume the smallest amount of O_2 per mg of cell volume, whereas the smallest erythrocytes (of *Hyla*) consume the largest amount of O_2 per mg of cell volume. There is therefore some reason to assume that the cell size may have an adaptive value. The adaptive values of variations in the amount of DNA are, however, much less convincing. The hypothesis that the redundancy of genetic information resulting from the increase in the amount of DNA may confer some insurance against mutagenic agents[4] disagrees with the findings of Sparrow, Schairer and Sparrow[8], who have demonstrated that the radiosensitivity of plant cells is directly proportional to the DNA content and to the volume of the nucleus. It could be supposed that the animals with large amounts of DNA per nucleus can produce more proteins; this was, however, not confirmed by Comings and Berger[22]. These findings also do not support the hypothesis[2] that the redundant DNA allows a slow accumulation of genome variability which may later be brought into play during a period of evolutionary explosion and diversification. Instead, it seems that natural selection acts chiefly on the size of the cell, although the correlated change in the amount of nuclear DNA may be the initial modifier of the genotype.

The following account of the changes in the amount of DNA in vertebrate nuclei is proposed. The invasion of estuaries and later of fresh water by the ancestors of vertebrates induced an increase in the cell size, accompanied by an increase in DNA. The tendency in vertebrate evolution turned later towards an increase in metabolic rate. Among aquatic vertebrates this trend is most pronounced in the teleosts. The decrease in cell size resulted in a decrease in the amount of DNA. The invasion of land by early tetrapods was probably accompanied by a decrease in metabolism and an increase in the size of the cells.

Only later was there a trend toward an increase in metabolism. Morescalchi[23] thinks that the evolution of anurans was accompanied by a reduction in the number of chromosomes. The erythrocytes of the primitive *Sphenodon punctatus* are larger than erythrocytes of all present day reptiles[24]. Birds have the highest metabolism accompanied by the smallest cell size and by the smallest amount of DNA per nucleus.

The development by Dipnoi of a resistance to high concentrations of urea in the blood was paralleled by an increase in the size of the cell and in the amount of DNA per nucleus. There is probably a similar reason for the very high amount of DNA in nuclei of Urodela, although the largest cells are found in such animals as *Amphiuma* and *Rhyacotriton*[25] which are not known to accumulate urea, but in *Scaphiopus* the amount of DNA in the nucleus is very low[1], though this toad can accumulate urea to a considerable extent[26].

In attempting an explanation of this, one comes against the problem that not enough is known about the factors that influence the size of the cell and the amount of nuclear DNA. It has been observed[9] in plants, for example, that there is a correlation between the amount of DNA in the genome and in the duration of mitotic cycle, and this also seems to be the case in some animals. According to Astaurov[27], the rate of mitosis in triploid and tetraploid silkworms is decreased in inverse proportion to the increase in the number of chromosomes and the size of the cell. Dettlaff[28] has calculated that at a temperature of about 18° C the duration of the mitotic cycle during the early stages of segmentation is 105 min in *Ambystoma*, 90 min in *Triturus*, 60 min in *Rana* and 50·3 min in *Acipenser*. This value is 31 min in *Misgurnus*[29]. These data seem to agree with the assumption that the duration of the mitotic cycle during the development of fishes and amphibians is influenced by the amount of nuclear DNA. Such correlation, however, is absent in birds and mammals. The strong selection pressure for the shortening of embryonal development, which in *Scaphiopus* takes place in temporary pools, is therefore the probable reason for the small amount of DNA in this genus.

I conclude that the differences in the amount of nuclear DNA in vertebrates are a result of differences in the cell size. Changes in the cell size during evolution have resulted from adaptations to various environments and to different modes of life. Big cells and a large amount of DNA per cell are correlated with a low level of metabolism and with the ability of cells to tolerate considerable variations in the composition of body fluids. Small cells and a small amount of DNA in the nucleus characterize groups with a high metabolism and with a precise regulation of their internal environment.

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Evidence for Epitaxy in the Formation of Collagen and Apatite

A NUMBER of structural relationships between the organic and inorganic components of bone are known from X-ray diffraction and electron microscopy studies. The interrelationships have led to a theory of mineralization involving the process of epitaxy, that is, the orientated overgrowth of bone mineral on certain well crystallized areas of the organic matrix. This differs in principle from theories of mineralization which assign no active role to the organic matrix in the mineralization process. Epitaxy clearly requires some structural symmetry, but considerable mismatch seems to be permissible in the process of crystal seeding. Results reported here indicate a structural symmetry in collagen and apatite in the presence of copper ion and support the concept of epitaxial growth; areas of identical crystal order on apatite and the organic substrate have been demonstrated as would be expected if epitaxy were a relevant mechanism.

In the technique used, the essentially diamagnetic substances to be studied are labelled with an inorganic paramagnetic ion. The ion is detected by electron paramagnetic resonance (EPR), and from an analysis of the shape of the signal the crystal field symmetry, if any, at the ion site is inferred. The cupric ion, Cu(II), was chosen as the labelling ion because (*a*) it has a known affinity for the substrates studied[1], (*b*) its hyperfine lines are sensitive to the local crystal fields[2], and (*c*) there have been previous studies involving Cu(II) bound to a variety of substrates which provide a basis for comparison with the present results[3–7].

Clinically normal human tendon was obtained during surgery and stored in an air-dried state until studied. Bovine tendon and animal skin gelatine were purchased. Human bone mineral was extracted by refluxing whole cortical bone pieces approximately $2 \times 3 \times 5$ mm in ethylenediamine for 72 h, then washing for 24 h in distilled deionized water at room temperature. Bone collagen was prepared by treating pieces of cortical bone with either 1·0 M formic or 1·0 M HCl for 72 h.

Bone mineral was exposed intact (that is, as the same crystalline aggregate as the original sample of refluxed bone) to solutions containing known concentrations of Cu(II). The ratio of the weight of solid to volume of solution was about 500 mg/l., and the standard exposure time for all materials was 24 h. The samples were rinsed, air-dried and reduced to powder. Human and bovine tendons and bone collagen were minced with scissors to a length of about 1 mm. After treatment with Cu(II) at a solid to solution ratio of 200 mg/l., they were rinsed and dried in the same way as the mineral. Cu-gelatine films were prepared by evaporating to dryness 5 per cent gelatine solutions made 0·068–0·83 mM with respect to Cu(II).

The native materials not treated with Cu(II) solutions exhibited either no resonances[1] or weak lines at $g = 2{\cdot}0$ which did not interfere with the present analysis[8–10].

After immersion in solutions containing Cu(II), bone mineral exhibited a well defined Cu(II) EPR spectrum (Fig. 1*a*). For solutions in the range 0·034 mM to 0·350 mM with respect to Cu(II) the spectrum is sharp and distinct and the overall line shape remains constant. As the molarity is raised above 0·35 mM the low field lines are less resolved and the main absorption peak becomes featureless. These results do not depend on the anion. Equimolar solutions of the sulphate, nitrate and chloride of Cu(II) yield identical spectra. Cupric ions absorbed on collagenous structures from solutions in the range 0·008 to 0·080 mM yield an EPR spectrum similar to that of copper labelled bone mineral (Fig. 1*b*). The set of four constants of the spin Hamiltonian which characterize each resonance are, within experimental error, identical for the two materials; thus the results indicate that there are sites of the same symmetry on each material. To give some idea of the spread in the spin Hamiltonian constants describing absorbed Cu(II), some representative values from the literature are given in Table 1. The virtual identity of the constants for bone mineral and collagen as compared with the spread in values shown in Table 1 is what is meant by "sites of the same symmetry" on collagen and bone mineral.

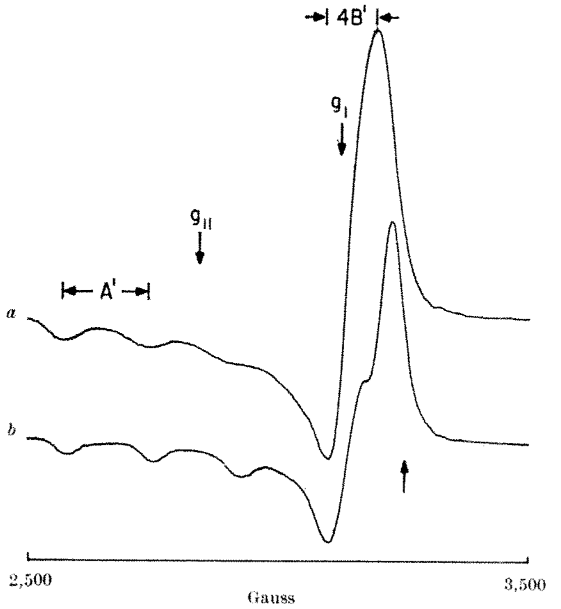

Fig. 1. EPR spectrum from (*a*) bone mineral, (*b*) tendon collagen after treatment with cupric ion. The arrow denotes the position of $g = 2{\cdot}00$.

We find no difference between the various collagen structures and gelatine with respect to the short range order about artificially added copper sites (Table 1). This seems to indicate that the secondary protein structure is not an important factor in determining the site crystal field symmetry.

Table 1. REPRESENTATIVE VALUES OF THE CONSTANTS IN THE HAMILTONIAN WHICH DESCRIBE THE SPECTRUM OF THE ABSORBED CUPRIC ION

	$g_{\perp}$	$g_{\parallel}$	A^*	B^*	Ref.
Laccase	2·048	2·197	90	—	6
Ceruloplasmin	2·056	2·209	80	—	6
Glass	2·06	2·32	157	23	3
Collagen, apatite, gelatine	2·07	2·27	170	< 20	—
DNA, RNA	2·08	2·35	153	< 30	7
'Dowex-50'	2·099	2·40	128	—	5

* $(\times 10^{-4}\ \text{cm}^{-1})$.

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Relationship between Mitosis and the Ordered Structure of the Stratum Corneum in Mouse Epidermis

THE cells of the stratum corneum of mammalian skin, although usually regarded as being randomly arranged, have been shown to be aligned in regular columns[1]. This arrangement was suggested to result from a regular pattern of cell migration from the basal layer. The available evidence, however, links cell migration to cell division and suggests that cell division is a random process[2].

The method previously used to display the columnar structure involved the expansion of the stratum corneum with a solution of sodium hydroxide during which the deeper layers of the epithelium were destroyed[3]. It has been found that acid solutions are as effective as sodium hydroxide for expansion of the stratum corneum, but they cause less damage to the deeper strata, and furthermore the damage caused by either agent is greatly reduced by a brief period of fixation before expansion. This has made possible a study of the position of mitosis in relation to the structure of the stratum corneum, and preliminary results suggest that there is a functional arrangement of the cells of the basal layer corresponding to the overlying columns.

We used the epidermis of the mouse because it consists of very few cell layers and has a particularly precise

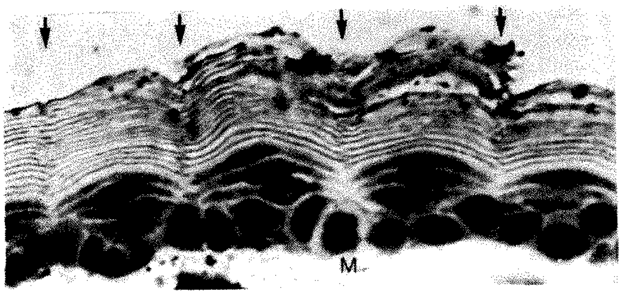

Fig. 1. 5 μm cryostat section of mouse ear epidermis after expansion in 0·1 M NaOH to display the junctions of the columns of squames in the stratum corneum (arrowed). The position of the mitotic figure (M) can still be identified. Alcohol fixed, stained 0·1 per cent methylene blue.

columnar arrangement of squames. Adult male mice were treated with 'Colcemid' (Ciba), 0·2 mg/100 g body weight for 6 h, to arrest dividing cells at metaphase. Cryostat sections, 5 μm thick, were fixed for 5 min in 70 per cent alcohol and treated either by staining in 0·1 per cent methylene blue followed by expansion in 0·1 M NaOH, or by expansion in 0·1 per cent methylene blue acidified to *p*H 4 with HCl. On 346 photographs of mitosis (Fig. 1) printed at a standard magnification, the outline of the individual squames of the columns in the stratum corneum could be seen clearly. The position of mitosis in relation to the columns was obtained by considering the basal region beneath each column to be divided into four regions of equal size. Mitoses occurring in either of the two outer quarters were classified as those related to the squamous junctions, and those occurring in the inner quarters related to the central region of the columns. The midpoint of the interdigitation of the lowest squames was used to define the position of the junctions between the columns, and the centre of each mitotic figure was classified as lying beneath an inner or outer quarter depending on the ratio m/s (Fig. 2).

If cell division occurs randomly among basal cells, it is to be expected that a similar number of mitotic figures would be seen in inner and outer quarters, for these represent equal lengths of the basal layer. We found 193 mitoses in the outer quarters and 153 in the inner quarters. The χ^2 test applied to these figures gave $P < 0\cdot05$, which suggests that mitosis has a tendency to occur beneath the junctional region of the overlying squames.

The columns of squames of the epidermis of the mouse ear, when viewed from the intact surface, are irregularly hexagonal with a diameter of about 30 μm. Sections 5 μm thick cut at right angles to the surface of such material pass through the central region of some columns but only through the peripheral region of others, and this is reflected in the width of the cut surface of each column seen in the sections. A width was chosen which divided the columns into two groups of approximately equal numbers, group 1 with a wide cut surface, and group 2 with a short cut surface. On the hypothesis that there is a tendency for mitosis to occur in the junctional region, the central area beneath each column should contain fewer mitoses. It is then to be expected that the group 1 sections will include this central region and show a greater tendency for mitosis to occur in outer quarters, and that group 2 which pass through the junctional region only would have an essentially random arrangement. We found that there was a greater tendency for mitosis to occur in the outer quarters of group 1 ($P < 0\cdot01$) and that there was no significant difference between the inner and outer quarters of group 2. This supports the hypothesis that mitotic activity is occurring principally beneath the periphery of each overlying column.

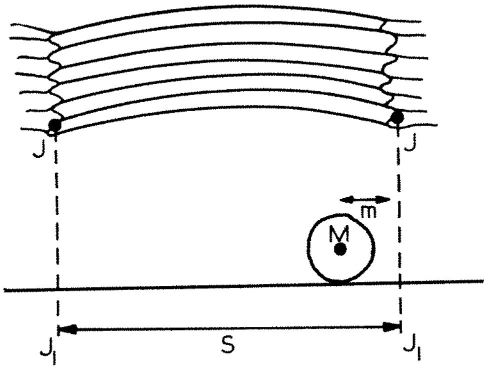

Fig. 2. Diagram of the method for determining the position of a mitotic figure in relation to the overlying structure. J, Midpoints of interdigitation between adjacent columns. Lines JJ_1, drawn through points J perpendicular to the line connecting them, define the basal region lying beneath the column of squames. S, Width of cut surface of column; m, distance of the centre of the mitotic figure (M) from the nearest junction.

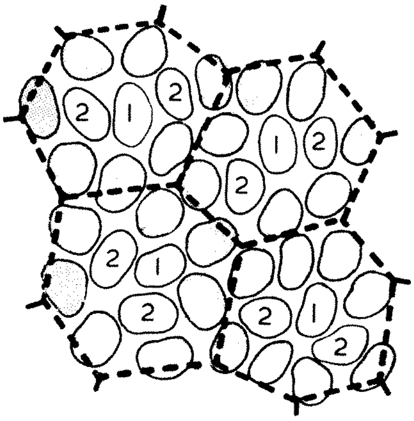

Fig. 3. A hypothetical pattern of basal-cell behaviour. Unit groups of basal cells lie beneath each polygonal column of squames. The rings of mitotically active cells (stippled) lying beneath the squamous junctions (broken line) produce differentiating cells (2) which move centrally to migrate into the stratum spinosum from the centre of each group (1).

If the columnar arrangement of squames of the stratum corneum results from some physical or cell-contact property during cell flattening, the regular stacking of the postmitotic differentiating cells of the stratum spinosum (Fig. 1) could result in a variation of chalone concentration in the basal layer. This would produce areas of relatively high and low mitotic activity (W. S. Bullough, personal communication). On the other hand, regular stacking could result from an intrinsic pattern of basal cell behaviour. Taken together with the finding that emigration from the basal layer is restricted to the oldest G_1 cells in the vicinity of mitosis[4], our results suggest that basal cells can be grouped into functional units, in which cells with mitotic potential lie in a ring at the periphery of each unit and produce differentiating cells which pass centrally to migrate into the stratum spinosum from a position beneath the centre of each overlying column (Fig. 3).

Table 1. DISTRIBUTION OF MITOTIC FIGURES

	Sections cut through		
	Central area	Edge of column	Total
Outer quarters	108	85	193
Inner quarters	71	82	153
χ^2	$P < 0\cdot01$	N·S	$P < 0\cdot05$

The presence in the basal layer of functionally separate unit groups of cells may prove to be a natural feature of the physiology of certain epithelia. It would imply a level of organization exhibiting finer spatial control than has previously been described in this tissue and would be important to an understanding of the control of epithelial growth both in normal and abnormal states.

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Nuclear Pockets and Projections in Fish Melanoma

NUCLEAR pockets (blebs or loops) and nuclear projections have recently been reported in various lymphoid cells of healthy[1,2] and leukaemic[3,4] human subjects, in Burkitt tumour lymphoblasts[5] and in thymocytes[6,7]. They have also been observed in myeloid cells of amphibians[8] and in lymphoid cells of birds and cyclostomes[9].

We report here the observation of similar nuclear structures in melanotic melanomas and amelanotic albino melanomas of platyfish-swordtail hybrids. The melanomas were fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded in 'Epon 812'. Thin sections stained with lead citrate were examined in a Philips 'EM 300' electron microscope.

Ultrastructural examination of the melanomas revealed that among small, highly dendritic melanocytes there are some large melanosome-containing cells possessing highly polymorphic nuclei (Fig. 1). Most of these nuclei show one or more nuclear pockets (Fig. 2) and/or projections (Fig. 3). The nuclear pockets are formed of a thin sheet of chromatin bounded on both sides by the nuclear envelope. They resemble those observed by other investigators in various lymphoid cells[1-9]. Frequently the cytoplasm surrounded by the chromatin sheet contains various organelles—for example, melanosomes (Fig. 2), endoplasmic reticulum or mitochondria (not illustrated). The nuclear projections are usually composed of a thin envelope-bounded sheet of chromatin with a dilation at its end (Fig. 3). The nuclear projections themselves often contain nuclear pockets (as indicated by arrows in Fig. 3). Frequently two nuclear projections can be seen meeting one another (Fig. 4), thus supporting the scheme for pocket formation postulated by Törö and Oláh[6] and later by Sebuwufu[7].

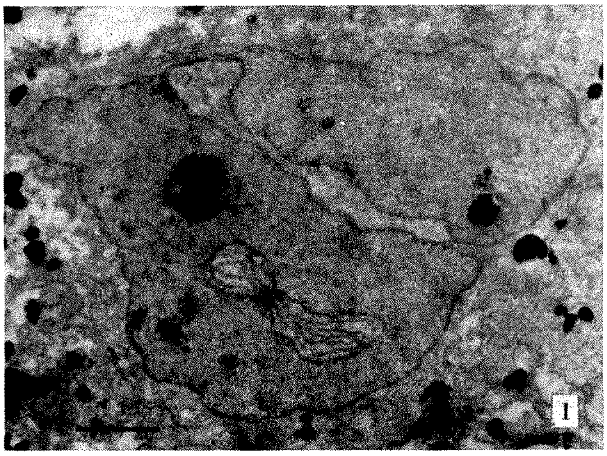

Fig. 1. Polymorphic nucleus in a melanotic melanoma cell. The nucleus contains a cytoplasmic area filled with rough endoplasmic reticulum.

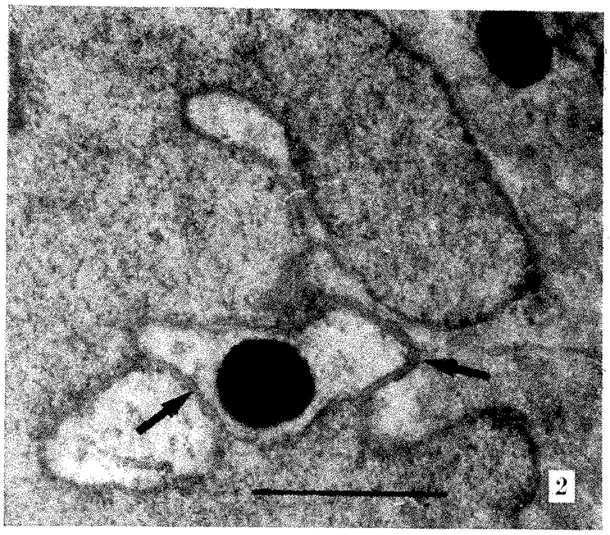

Fig. 2. Nuclear pockets in a melanotic melanoma cell. Arrows point to envelope-limited sheets of chromatin which surround a melanosome.

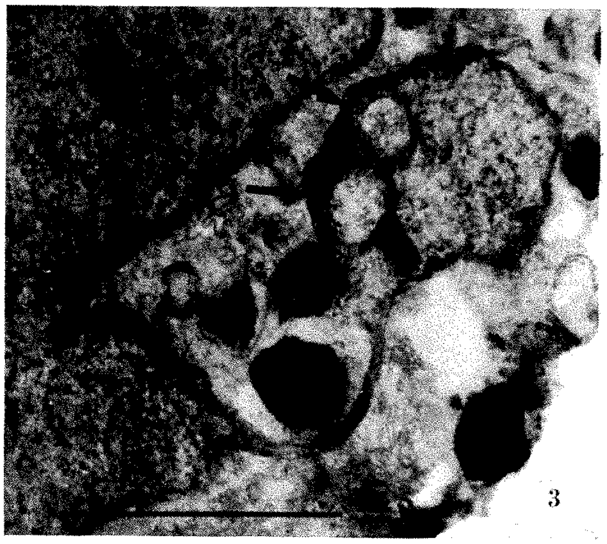

Fig. 3. Nuclear projection in a melanotic melanoma cell. Arrows indicate nuclear pockets.

Occasionally we have observed nuclei in melanoma cells containing large envelope-bounded cytoplasmic areas (Fig. 1) which represent deep indentations or perhaps wider nuclear pockets in another cross-section. The cytoplasm inside the nucleus is usually filled with several structures—for example, rough endoplasmic reticulum (Fig. 1), myelin figures, or phagocyte-like vacuoles which degrade melanosomes, or premelanosomes, in the case of albino melanomas. The latter structures have been found to be characteristic of melanocytes in platyfish-swordtail melanomas[10].

We can give no satisfying interpretation of the existence of nuclear pockets and projections. Their occurrence in melanoma cells and, as recently reported, in tumoral epithelial and connective tissue cells[11] and in follicle cells[12] may indicate that these structures are not only characteristic of cells of the vascular system but are more widely distributed. We conclude from our electron microscopic studies that nuclear pockets and projections do not occur in the small dendritic melanocytes, the nuclei of which are ovoid or round in shape. This finding suggests that in large cells nuclear pockets and projections, as well as highly polymorphic nuclei, may have the function of

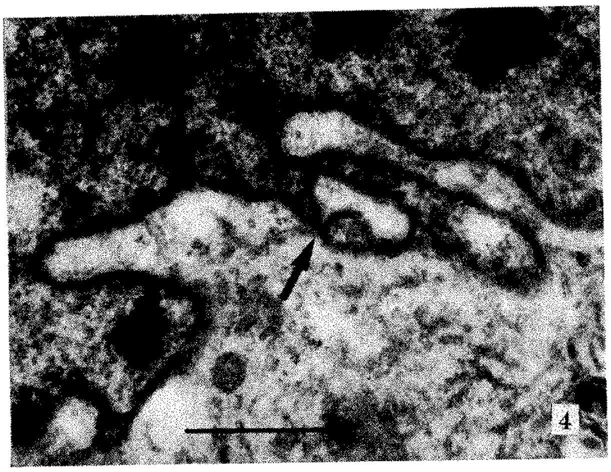

Fig. 4. Nuclear projections in an amelanotic albino melanoma cell. Arrow indicates where the projections seem to undergo pocket formation.

increasing the area of chromatin in contact with the cytoplasm (see refs. 2 and 9).

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Unique Vascular Relationship in the Human Umbilical Cord

THE reactivity of both perfused and isolated segments of human umbilical vessels to various pharmacological agents has already been reported[1-5]. Without exception, serotonin (5-hydroxytryptamine) has been found to be a potent constricting agent of both umbilical artery and vein. In perfusion studies, however, arteries and vein were generally not perfused simultaneously or, in cases where they were, drugs were administered only to the arterial perfusing fluid.

We have perfused both the arteries and the vein simultaneously in 10 cm segments of human umbilical cords, and injected drugs into both the arterial and venous perfusing fluids, which consisted of Krebs–Henseleit solution, equilibrated with oxygen: carbon dioxide (95 per cent : 5 per cent), in physiological pressures (10 mm of Hg in the vein, 55 mm of Hg in the arteries), although the flows obtained (12 ml./min in the vein, 6 ml./min in each artery) were considerably less than those found *in utero*[6]. Decreased flow is a familiar problem in studies of isolated perfused placentae and umbilical cords[2]. Perfusion pressure was measured in the vein using a Statham pressure transducer. Spirally and longitudinally cut strips of umbilical artery and vein were examined in a tissue bath, and the muscular activity was recorded isotonically on a kymograph.

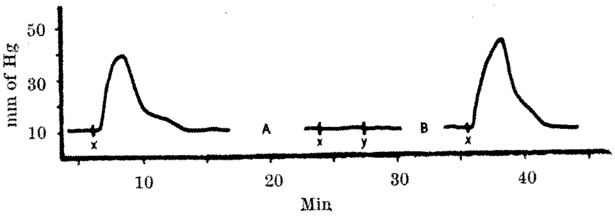

Fig. 1. Pressure changes from serotonin in perfused umbilical vein before and after insertion of a stainless steel rod into one artery. Serotonin was injected as a bolus at x (10^{-8} g) and at y (10^{-7} g). The rod was inserted during interval *A*. Note that changes in perfusion pressure could not be obtained with serotonin while the rod was in place. Removal of the rod (in interval *B*) was followed by a return of the response to intravenous serotonin.

Very small doses (2×10^{-9} to 1×10^{-8} g) of serotonin injected as a bolus in the venous perfusing fluid caused increases in venous perfusion pressure while the arteries were present in the segment of cord, whether or not the arteries were perfused. Administration of as much as 10^{-5} g of serotonin to the arterial perfusing fluid did not alter venous perfusion pressure, although arterial flow was terminated rapidly. When the arteries were dissected sharply from thirty segments their response to intravenous serotonin was abolished. This response was also abolished in twenty cords when a stainless steel rod was inserted through one of their arteries, thus uncoiling the vessels from around the vein. When the rod was removed the system recorded its sensitivity to serotonin, which indicates that the contractile mechanism involved was undamaged by the procedure (Fig. 1).

We then investigated whether removal of the arteries from the segment of cord had damaged the contractile mechanisms of the vein. Spirally and longitudinally cut strips of umbilical vein were prepared from unperfused segments of the cord, from segments perfused with the arteries in place, and from segments perfused after removal of the arteries. In every case, the venous strips prepared from these three kinds of tissue had identical sensitivity to serotonin in the tissue bath, each contracting at a threshold dose of serotonin of 10^{-9} g/ml.

Longitudinally cut strips of human umbilical artery contracted in response to serotonin, with a threshold concentration of 1 to 5×10^{-9} g/ml., representing a unique component of human arterial tissue. We suggest that the contraction of the longitudinal muscle fibres in the arteries, which coil around the vein in the human umbilical cord, was responsible for the increase in venous perfusion pressure after addition of serotonin to the venous perfusing fluid. That this may be a unique mechanism in man, or at least in the higher primates, is suggested by the observation that sheep umbilical cords contain two arteries and two veins with little coiling of one about the other, and the apparent absence of functional longitudinal muscle in either vessel[7].

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Fitting Sine Curves to 24 h Urinary Data

In studies of biological rhythms it is often desirable to obtain an objective estimate of the phase and amplitude of a suspected rhythm from observation recorded over a single cycle, such as a single period of 24 h. The usual technique is to attempt to fit the observations to a sine curve. Most techniques of fitting a sine curve are applicable only to a series of points and, for conventional methods, the points must be uniformly spaced in time. Many biological data, especially of renal excretion but also of respiratory or heat exchange, represent the average value of the variables since the previous sampling. The sampling intervals are often irregular and are correctly represented graphically by a histogram, though in published work they are often presented as points at the midpoint of the collection period. This does not cause much distortion if the time intervals are short and equal, but a gross distortion of the actual behaviour is introduced when, for example, there are long collection periods of urine as in a night's sleep.

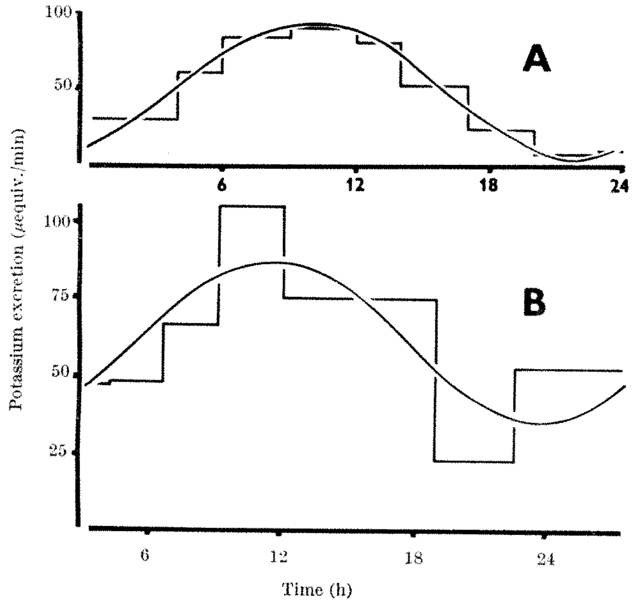

Fig. 1. Rate of potassium excretion measured in a series of urine samples over 24 h. and the sine curve which best fits the data. *A*, Artificially constructed to illustrate perfect fit. *B*, Experimental data, with best fitting sine curve calculated by computer.

We have attempted to fit sine curves to such data in three ways. The first method, which makes hand calculation feasible, was to calculate the excretion rate corresponding to each 2 h period over 24 h and fit a sine curve to the twelve equally spaced mid-points thus derived[1,2]. More recently we have used a computer program, hereafter referred to as method *A*, which fitted a sine curve to a large number of equally spaced points. Each urine sample was treated as if it were a series of identical consecutive samples, each covering the longest time interval that was an integral fraction of every sampling period. For example, if every sample was produced exactly on a quarter-hour, the whole 24 h would be treated as ninety-six successive samples, a sleep of 7·5 h being then regarded as thirty identical samples.

Both these treatments, even if approximately correct in their predictions, involve some falsification of the experimental evidence. The production of thirty identical successive urine samples would be evidence against a sinusoidal oscillation. The following considerations suggest a more satisfactory approach.

If the abscissa of a histogram is time, and the ordinate is the rate of excretion of a urinary constituent, the area beneath each rectangle of which the histogram is composed represents the amount excreted, such as a volume of urine or milliequivalents of an electrolyte. In the fitted sine curve this amount is represented by the integral of the sine curve over the appropriate time interval, and the fit of the curve is indicated by the accuracy of prediction of this area. It is thus theoretically possible, even if infinitely improbable, to obtain a perfect fit, as in the artificially constructed example in Fig. 1*A*. The criterion here adopted to assess departure from perfect fit is to take the differences between predicted and observed areas, square them and sum the squares, and we have written a computer program, referred to as method *B*, which calculates the parameters of the sine curve of period 24 h that will reduce this sum of squared deviations to a minimum, consistent with an initial condition that the mean rate of excretion, and consequently the total excretion over the 24 h as predicted from the sine curve, must be equal to the observed value.

Fig. 1*B* shows an example of this procedure applied to experimental data of rate of potassium excretion sampled over 24 h. Because this process of calculating the deviation from a sine curve bears some formal similarity to the measurement of variance, we have adopted a similar statistical procedure:

$$a = \frac{\text{sum of squared deviation from the curve}}{n-3}$$

$$b = \frac{\text{sum of squared deviations from mean}}{n-1}$$

$$Q = a/b, \text{ where deviations are of area}$$

The sum of squared deviations of determined areas from those predicted from the sine curve is divided by the number of urine samples minus three, because three parameters are needed to define a sine curve. This quantity is a measure of goodness of fit, and is then divided into a quantity bearing a similar formal resemblance to a total variance, namely, the sum of squared differences between determined areas and those predicted from uniform urinary excretion over the whole 24 h, divided by the number of urine samples minus one. The ratio thus obtained, while not strictly a variance ratio, conveys similar information about the goodness of fit of the sine curve.

This method may be made clearer by Fig. 2 and Table 1, in which the same experimental data for potassium excretion are plotted with the sine curves fitted by methods *A* and *B*. In Fig. 2*A* the deviations from the mean excretion over 24 h are shaded, and their numerical value and the sum of their squares are shown in Table 1. In Fig. 2*B*, the deviations from the sine curve are similarly shaded and their numerical values and sum of squares, together with those derived from method *A*, are also shown in the table. It will be seen that for sample 3 the deviation is very small, for it consists of nearly equal positive and negative portions. The table further shows that both sine curves give a much better prediction of potassium excretion than does the 24 h mean, and that method *B* is better than method *A*.

The program could readily be modified to be applicable to data covering an integral number of cycles, or with more difficulty to a non-integral number. With a much greater expenditure of computer time it could be extended to test the fit to a series of periods, as is sometimes required in "free-running" experiments, and thus to search for some outstanding frequency in a long series of "noisy" data. It could also be extended to fitting periodic curves containing higher harmonics.

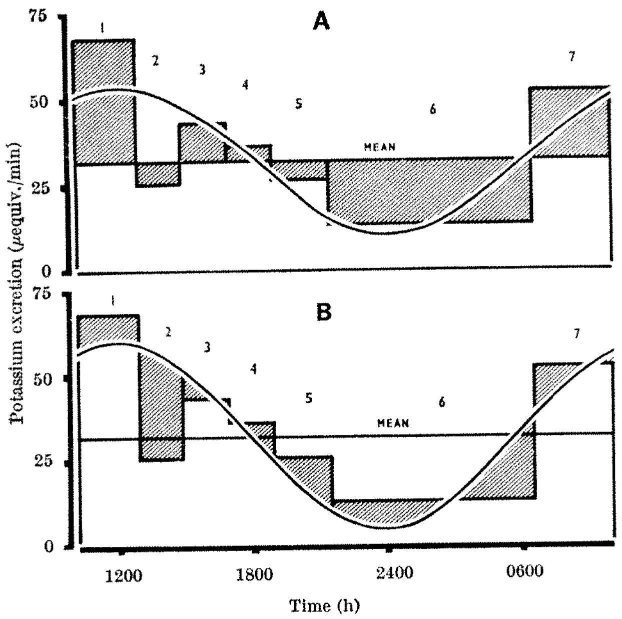

Fig. 2. Potassium excretion over 24 h observed experimentally, with best fitting sine curves calculated by methods *A* and *B*. Shaded areas represent, in milliequivalents of potassium, the deviation of observed potassium excretion in each urine sample from *A*, mean excretion rate over 24 h, and *B*, excretion predicted from the sine curve.

Table 1. CALCULATION OF DEVIATIONS IN FIG. 2

Urine sample	K^+ content (milli-equivalents)	Deviation from mean 24 h rate (milli-equivalents)	Deviation from sine curve *A* (milli-equivalents)	Deviation from sine curve *B* (milli-equivalents)
1	10·478	+5·593	+2·351	+1·424
2	3·060	−0·712	−3·026	−3·498
3	5·184	+1·402	−0·038	−0·152
4	4·320	+0·538	+0·349	+0·624
5	3·870	−0·857	+0·707	+1·477
6	7·160	−10·330	−2·136	−0·496
7	11·308	+4·375	+1·791	+0·622
$s[d^2]$		160·628	23·077	17·491
Degrees of freedom		6	4	4
Variance		26·771	5·769	4·373
Variance ratio			4·640	6·122

The idea behind method *B* arose out of a discussion with Professor J. F. Adams, and we thank Dr A. V. Reader for assistance with the computer program.

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Stimulation of Glycerol Production in Fat Cells by Cholera Toxin

CELL-FREE filtrates of cultures of *V. cholerae* contain a toxin which produces fluid accumulation in ligated rabbit ileal loops[1] and increases capillary permeability in rabbit skin[2]. In isolated rabbit ileal mucosa, it induces chloride secretion and inhibition of normal, non-glucose mediated sodium absorption[3]. The effects on ion fluxes are similar to those produced by theophylline or cyclic adenosine 3′,5′-monophosphate (cAMP) and, in addition, the effects of cholera toxin on gut fluid loss are mimicked by agents that can increase tissue cyclic AMP levels[4]. We therefore investigated the effects of the toxin on fat cells in which accumulation of cAMP resulting either from increased synthesis (for example, with epinephrine) or from decreased degradation (for example, with theophylline) results in stimulation of lipolysis[5]. As reported below, cholera toxin increases glycerol production by fat cells. These studies have provided the basis for a simple, sensitive assay for choleragen.

Table 1. INHIBITION OF THE TOXIN EFFECT ON GLYCEROL PRODUCTION

Exp. No.	Conditions	Toxin μg/ml.	Percentage inhibition of toxin effect Hour 2	Percentage inhibition of toxin effect Hour 3
1	Plus cyclic 3′,5′-AMP, 1 mM	40	55	30
2	Plus ethacrynic acid, 1 mM	96	100	100
	3 mM		100	100
	1 mM	240	100	100
	3 mM		100	100
3	Minus Ca^{2+}	40		84
	Minus Mg^{2+}			48
	Minus Ca^{2+} and Mg^{2+}			91
4	Minus K^+	200	59	71
	Minus Ca^{2+} and Mg^{2+}		69	68
	Minus Ca^{2+}, Mg^{2+} and K^+		91	94

In exps. 1 and 2, cells were washed and incubated in Krebs–Ringer phosphate medium with additions as indicated. In exp. 3, cells were washed in medium from which Ca^{2+} and Mg^{2+} were omitted and incubated in this medium with and without the addition of Ca^{2+} and/or Mg^{2+} at final concentrations equal to those in the complete medium. Exp. 4 was carried out in a similar fashion except that Ca^{2+}, Mg^{2+} and K^+ were omitted from the medium used for washing and added to the incubation medium where indicated. Samples (0·5 ml.) of cells plus medium were taken after 0, 1, 2 and 3 h of incubation for determination of glycerol. Percentage inhibition of the toxin effect during the second and third hours of incubation was calculated from the mean glycerol production during these periods for two or three incubations under each set of conditions.

Procedures for the preparation and incubation of fat cells and for the determination of glycerol are described in an earlier publication from this laboratory[6]. Krebs–Ringer phosphate medium containing bovine serum albumin, 30 mg/ml., was used for all incubations and for washing cells except where indicated (that is, Table 1, experiments 3 and 4). Each incubation contained 15–40 mg fat cells in a total volume of 2·5 ml. Lyophilized supernatant fluid from cultures of *V. cholerae* Ogawa B 1307 (Wyeth Co.) grown according to Craig[7] was used except where noted. Activity is expressed in terms of dry weight of the lyophilized material.

A significant increase in glycerol release could be demonstrated after 3 h incubation with 8×10^{-6} g/ml. of the Wyeth crude toxin. Even with high concentrations of toxin (4×10^{-4} g/ml.) there was no stimulation of lipolysis during the first hour of incubation. After the first hour the rate of lipolysis increased progressively. Fig. 1 illustrates the time course of the reaction. This experiment was terminated at 3 h. In other experiments the rate of lipolysis continued to increase during the fourth hour. This was demonstrable, however, only when incubations were carried out with small amounts of cells and/or with low concentrations of toxin, for accumulation of free fatty acid beyond about 6 moles per mole of albumin inhibits lipolysis[8]. Thus, in the experiment summarized in Fig. 2, the rate of glycerol production during the third hour was greater than that during the second hour with concentrations of toxin below 1×10^{-4} g/ml., but above this level was no longer proportional to toxin concentration. During the second hour the rate of glycerol production was proportional to toxin concentration over the entire range tested.

As shown in Fig. 1, the addition at the end of the second hour of either prostaglandin E_1 or insulin at concentrations that inhibit hormone stimulated lipolysis[5,9] inhibited the effect of toxin during the subsequent hour. Cyclic AMP inhibits theophylline stimulated lipolysis in Krebs–Ringer phosphate medium[10] and likewise interfered with the effect of toxin (experiment 1, Table 1). Ethacrynic acid reduces gut fluid loss and the ion flux alterations produced by cholera toxin[11]. At concentrations of 1 or 3 mM, it completely inhibited the effect of toxin on fat cells (experiment 2, Table 1). In the same experiment, 1 mM ethacrynic acid inhibited the lipolytic effect of 2×10^{-8} g/ml. epinephrine to a lesser extent and only during the second 30 min of incubation. (The incubations with epinephrine were terminated at 60 min.) The effects of epinephrine and other lipolytic hormones are influenced by the ionic composition of the incubation medium. In experiment 3, Table 1, omission of either Ca^{2+} or Mg^{2+} significantly decreased the effect of 4×10^{-5} g/ml. of toxin. Omission of both divalent cations nearly abolished it. In experiment 4, Table 1, the effect of 2×10^{-4} g/ml. was decreased about 70 per cent in medium without Ca^{2+} and Mg^{2+}. The omission of K^+ similarly decreased the effect of toxin. In the absence of all three ions, that is, in all Na^+ medium, there was virtually no effect of toxin.

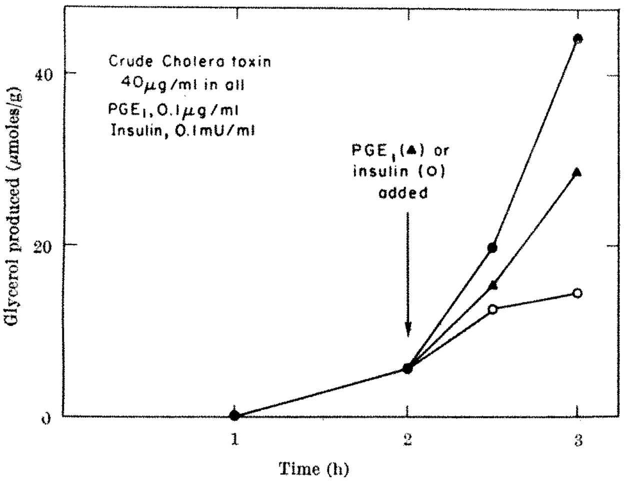

Fig. 1. Effect of crude cholera toxin on glycerol production. Crude cholera toxin was present throughout the incubation period. ●, Glycerol produced in the presence of toxin alone. Glycerol production in the absence of toxin (or with boiled toxin, 200 μg/ml.) was less than 5 μmoles/g/3 h (not shown). Points represent the mean of duplicate incubations that agreed within < 5 per cent.

Because the toxin preparations used in initial experiments were a mixture of many products of *V. cholerae*, it was necessary to ascertain whether the lipolytic response was due to the fluid transport active principle (choleragen) which causes fluid loss in gut. Boiling for 5 min inactivated the choleragenic toxin and also inactivated the lipolytic agent. Neither was dialysable through 'Cellophane' membranes. Concentrated cell-free filtrate from a strain of *V. cholerae* (strain In 569B) grown in conditions such that it did not produce detectable choleragen also had no effect on lipolysis. A highly purified choleragen[12], kindly provided by Dr Richard A. Finkelstein, significantly stimulated lipolysis at a concentration of $3{\cdot}5 \times 10^{-9}$ g/ml. A very similar but biologically inactive form of this toxin (choleragenoid) produced no lipolysis at 1×10^{-4} g/ml. Both of these materials produced a single band in polyacrilamide gel electrophoresis and elicited a single precipitin band when reacted with antisera which had been adsorbed with live *V. cholerae*[12]. The sensitivity of fat cells to cholera toxin is similar to that of skin and considerably greater than that of rabbit ileal loops. Antisera prepared against purified toxin abolished the lipolytic activity of choleragen.

The results of all our experiments thus far are consonant with the conclusion that it is the fluid transport active factor (choleragen) that stimulates lipolysis in fat cells. This, together with the fact that the stimulation of lipolysis is proportional to log-dose over a wide range of toxin concentrations, has made it possible to use the fat cell system as a rapid, sensitive assay for choleragen and cholera antitoxin (our unpublished experiments). The fat cell system, consisting as it does of a homogeneous population of free floating cells, may prove useful in investigation of the mechanism of action of choleragen which is still to be elucidated. The findings presented here, as well as the data from studies of effects of cholera toxin on the intestine[3,4], are consistent with the idea that choleragen may affect processes mediated by cAMP in tissues outside the gastrointestinal tract.

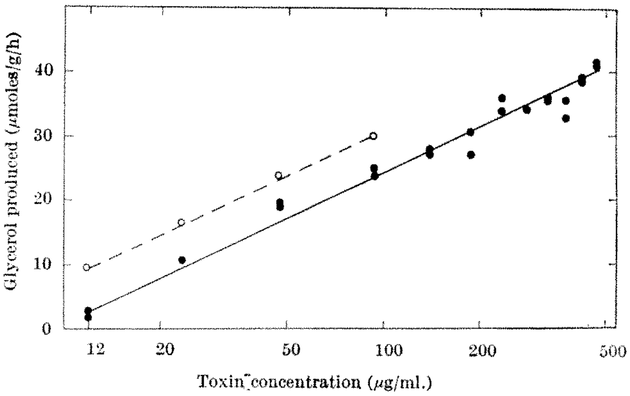

Fig. 2. Glycerol production as a function of concentration of crude cholera toxin. Values for glycerol produced during the second hour of incubation (●—●) represent data from individual incubations. Those for the third hour (○ - - - ○) are the means of the data from two incubations that agreed within < 5 per cent.

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Territorial and Courtship Songs of Birds

THE usual interpretation of passerine song is that it simultaneously attracts potential mates and repulses neighbouring territory holders[1]. The male black-throated green warbler (*Dendroica virens*, family Parulidae), however, sings two similar but distinct songs[2]: song *A* (Fig. 1*a*) chiefly in the presence of conspecific males and song *B* (Fig. 1*b*) chiefly in the presence of conspecific females. There seem thus to be separate songs for territorial defence (song *A*) and courtship and pair-bond maintenance (song *B*).

A natural experiment, in which males have no neighbouring territory holders of the same species, is created by several small islands off the coast of Maine. The birds are part of the same gene pool that controls birds on larger neighbouring islands that contain many pairs of the same species. If the songs are always sung in certain contexts song *A* will be infrequent among the isolated males in comparison to birds on larger islands. Further, song *B* might be relatively more frequent during the courtship phase but less frequent during the bond-maintenance phase when the mate is constrained to the small island. I report here the results of recording the frequencies of the two song types in two representative habitats throughout the breeding season.

Table 1. SIZE AND RELATIVE ISOLATION OF FORESTS ON SMALL ISLANDS

Name of island	Size of forest (ha)	Distance to forest on nearest island containing black-throated green warblers (m)
Crane	0·53	230 m
Indian	0·69	225 m
Jim's	0·49	500 m
Thief	1·50	1,070 m

The study was conducted on Hog Island (Todd Wildlife Sanctuary), Bremen, Lincoln Co., Maine, and small islands 0·3–7·3 km away from it (Table 1). Hog Is. is largely covered with a forest of red and white spruces (*Picea rubens* and *P. glauca*) 18 to 25 m high; the habitat is described in detail elsewhere[3]. The small islands (Jim's Is., Bremen; Indian Is. and Thief Is., Bristol, Lincoln Co.; Crane Is., Friendship, Knox Co.) all contain white spruce forests superficially similar to that of Hog Is., except that in several cases the trees are shorter, and that in all cases the habitat has a high proportion of edge. These habitats will be discussed in greater detail elsewhere.

We counted songs for periods of 10 min regularly throughout the season (usually every other day), always within the hours of 0900–1100 DST. From the middle of a small island it was possible to record all the songs. Data were collected from Indian Is. and Thief Is. in 1967, 1968 and 1969; from Crane Is. in 1967; and from Jim's Is. in 1968. Six males were studied; the same birds nested on Indian Is. and Thief Is. in both 1968 and 1969. The number of songs in 10 min was counted throughout the 1966 season from a randomly selected group of 10 birds on Hog Is. Periodic counts of songs were made on Hog Is. in 1969 and indicated no apparent differences from the complete 1966 data. All individuals counted on Hog Is. were apparently mated; all isolates were certainly mated.

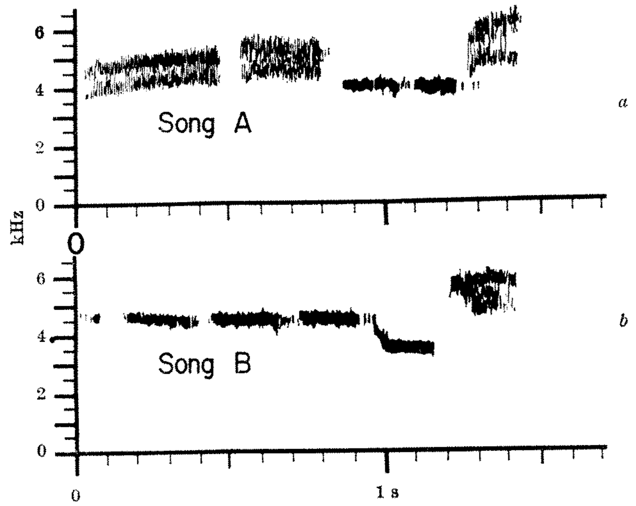

Fig. 1. Songs of the black-throated green warbler. Drawings of sound spectrograms.

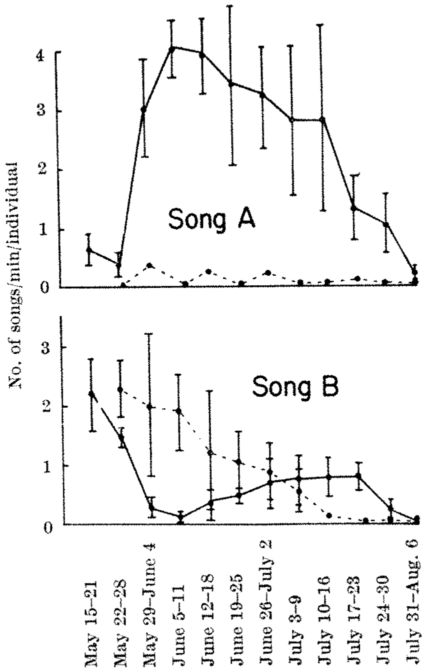

Fig. 2. Types of songs of male black-throated green warblers on Hog Island (———) and small islands (- - -). One standard deviation plotted.

The results appear in Fig. 2. Birds in large populations produced song *A* far more than isolated birds at almost any period of the season; but isolates gave more song *B* during the first half of the breeding season (and a much greater percentage of song *B*) than birds in large populations. During the last half of the season, song *B* came more from birds in large populations than from isolates. At almost all times the combined frequency of songs *A* and *B* from each bird in large populations was much greater than the frequency on small islands.

The first high frequency of song *B* occurred in the pairing period immediately after the birds arrived, with the greatest counts of song *B* at this time in both the large population and among isolates. After apparent pairing the frequency dropped precipitously in the large population, with a simultaneous increase of song *A*. In the isolates, however, song *B* remained dominant, although it declined continuously in frequency as the season progressed. If conspecifics are absent, more time should be free for other activities and, perhaps accordingly, the isolates gave a high (though declining) frequency of song *B* during the first part of the season.

The gradual but constant increase in song *B* among the large population after the early drop showed an inverse relationship to the concurrent decrease of song *A*. The basis for an almost continuous song *A* had probably declined somewhat by then. The increase of song *B* began at the peak of the incubation period, when there was little contact between sexes because only the females incubate[4].

The possible effect of congeners requires comment. In large populations three are present: magnolia (*D. magnolia*), myrtle (*D. coronata*) and blackburnian (*D. fusca*) warblers, as well as the smaller parula warbler (*Parula americana*)[3]. Myrtle and parula warblers were present on each small island studied (one pair of each species), but magnolia and blackburnian warblers never nested. The possibility thus arises that the missing species might affect the song patterns of black-throated green warblers, for the latter are not oblivious to the songs of congeners[2]. Although they did not nest, however, singing blackburnian warblers were observed three times on Indian Is. and in no instance did black-throated green warblers give song *A* in their presence. Similarly, singing magnolia warblers were observed twice on Thief Is. and once on Indian Is., and during these periods no song *A* was heard.

On the other hand, in 1969 a second male black-throated green warbler tried to establish territory on Thief Is. The resident male produced song *A* frequently when close contact was noted between the two birds (June 12–18, June 26–July 2 in Fig. 2), so that a resident male seems capable of singing this pattern in appropriate situations. Song *A* was recorded on Indian Is. between May 29 and June 4 in two different years; the resident male may have responded to an undetected male or, more likely, to songs from Loud's Is. across a 225 m gap of water and rocks. Loud's Is. supports a large population of warblers similar to that of Hog Is. When giving song *A* at these times the male from Indian Is. always situated itself on the side of the island immediately adjacent to Loud's Is.

In summary, song *A* occurred less often, and song *B* initially more often, among isolated males than among individuals in large populations. Further, among isolates song *B* became less frequent through the season until it was rarer than in large populations. My results suggest that the type of song was strongly regulated by situations experience rather than by heredity. They also strengthen the earlier hypothesis that the two songs act in different behavioural contexts.

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Semi-terrestrial Shrimp *(Merguia rhizophorae)*

IT is widely believed that there are no semi-terrestrial or terrestrial species of shrimps and prawns (order Decapoda: suborder Natantia)[1], but I now report that the shrimp, *Merguia rhizophorae* (Rathbun, 1900) (family Hippolytidae), is semi-terrestrial. It seems to have evolved behavioural adaptations which have allowed invasion of the terrestrial environment.

This shrimp was originally described from the State of Alagoas, Brazil, where it was found "on mangroves"[2]. Holthuis[3] redescribed and illustrated this shrimp from specimens collected in Suriname and determined its correct generic position, and his detailed notes support my observations on the semi-terrestrial habits of this species (see also his remarks on *M. oligodon*).

I first observed and collected *M. rhizophorae* in a mangrove area on the Atlantic coast of Panama in May 1969. A specimen was found under a dry piece of driftwood approximately 1 m above the high tide mark. When I tried to grasp it, the animal leapt onto some nearby mangrove roots, and moved up and down the roots and leapt from one root to another.

During July, I observed nineteen shrimps during the day and night, at high and low tides (representative specimens are in the US National Museum, No. 127510). All the specimens were found above the high tide mark among piles of driftwood, 0·3 to 0·5 m off the ground. The substrate, damp sand with mats of algae present, was probably wet by rainfall, seepage, or an occasional spring tide, normal tides not reaching this level. The shrimps were found under the driftwood during the day and out on the wood at night. They remained motionless when first observed, but when disturbed they leapt onto other pieces of driftwood and mangrove roots. The shrimp did not appear to orient towards the water while attempting to escape, and no specimens were seen in water nearby.

Four shrimps were placed on an unprotected cemented area for further observations. The air temperature was 31° C, wind velocity 12 m.p.h., on a partly cloudy day. The shrimps moved about in an apparently random manner. They walked in a forward direction using the last three pereiopods in a manner resembling insect locomotion. The abdomen was arched so that the body and telson were raised off the ground. Before movement, the elongate and spiniferous third maxillipeds were pressed together and extended in front of the shrimp. They were then lowered onto the substrate, and contracted. The shrimp climbed mangrove roots and wood in the same manner. The third maxillipeds were pressed together during the entire process and were re-extended onto the substrate when the shrimp became motionless. Shrimps, 10–20 mm in length, were able to leap in any direction over distances of 20–30 cm and 10–15 cm in height, the forward leaps being the longest and highest. Their motion was too rapid to determine to what extent flipping of the abdomen and using the walking legs played in leaping. The abdomen was arched high so that flipping the telson against the substrate probably began the leap, the legs then being used to direct it. The shrimps walked about until a shaded, protected area was located; then they became quiescent. They could be made to move by moving a small net to within a few centimetres of them. One specimen leapt thirty-six times during a 4 min period, sometimes without apparent provocation. Holding them in a moist beaker for 4 h did not affect their previously observed behaviour.

The general morphology of *M. rhizophorae* does not seem to be specially adapted for terrestrial habits. The eggs, however, are large (0·9 mm) compared with a closely related hippolytid *Hippolysmata wurdemanni* (0·28 mm). This suggests an abbreviated larval development, perhaps similar to *Thor* sp., another hippolytid which has a comparable egg size and undergoes metamorphosis within 48 h after hatching[4]. The pereiopods of *M. rhizophorae* are more robust than in most marine shrimps and are able to support the body on land (most shrimps, if they are able to move on land at all, drag their body). The eyes are large and may be adapted for a nocturnal existence because they possess a strong reflecting layer.

This shrimp's behaviour may be defensive. The animal seemed to avoid water, and the ones observed in Panama were never found directly on the ground. The waters surrounding the mangrove are heavily populated by predacious fish (*Lutjanus* sp.) while predacious crabs (Xanthidae) are on the ground. When pursued, the shrimp avoided these areas. The shrimp was motionless when first observed and relied on its cryptic attitude and coloration as a primary defence. Its secondary defence, leaping, was used only when the "predator" was very close. This extremely rapid leap was very effective. The shrimps can maintain this type of avoidance behaviour for more than 4 min. This is interesting because several brachyuran crabs which I examined could maintain an avoidance behaviour for only 40–90 s (M. H. Robinson, L. G. A. and B. Robinson, in preparation).

Merguia rhizophorae probably enters the sea occasionally, but only at certain times and for brief periods. My studies indicate that it remains in a moist habitat during the day and only comes out at night. It thus seems that *M. rhizophorae* avoids most problems accompanying terrestrial life through behavioural adaptations.

This work was carried out at the Smithsonian Tropical Research Institute while I was associated with the University of Panama. I thank my friends and colleagues for constructive criticism.

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Development of *Dracunculus medinensis* after Freezing

I BELIEVE this to be the first report of development to maturity of a parasitic helminth after prolonged storage at low temperatures.

Among nematodes the microfilarial larvae of *Wuchereria bancrofti* were stored at −25° C in heparinized blood[1], and a few of those of *Dirofilaria immitis* developed in the mosquito, *Anopheles quadrimaculatus*, after storage[2] at −68° C. Recently, development of a few microfilariae of *Brugia pahangi* to the infective third stage in *Aedes aegypti* has been reported after storage for twenty-nine days at −79° C in serum containing 5 per cent dimethyl sulphoxide[3].

Embryos of *Dracunculus medinensis* in distilled water have been successfully deep frozen for months[4] and, after thawing, 0·05 per cent of them developed to the infective third stage in *Cyclops leuckarti* (Claus)[5].

In my experiment one batch of larvae was obtained from a patient in Western Nigeria in May 1966 and another from an experimentally infected rhesus monkey in June 1969. They were cooled slowly (5° C/min) to −78° C in ampoules containing about 2,000 embryos per ml. of sterile distilled water without additives (glycerol and dimethyl sulphoxide were deleterious). Embryos were thawed quickly at 37° C and left for two hours at 30° C before being used for infecting *Cyclops vernalis americanus* (Marsh). Infected cyclops were maintained for fourteen days at 26° C and then kept for two hours at 37° C in normal saline containing 0·01 per cent HCl. Twenty-five infective third-stage larvae were released from cyclops infected with embryos stored for 6 months (1 per cent infection rate) and one larva from a cyclops infected with embryos stored for 40 months. This is a very low recovery compared with the 80–90 per cent infection rate and average of two larvae per infected cyclops found when using freshly obtained embryos[6]. The released larvae were injected intraperitoneally into a young male rhesus monkey (*Macaca mulatta*) and the remaining cyclops (probably containing a few more larvae) were given by gavage.

Male worms reach maturity after seventy to eighty days in the rhesus monkey (mean size 14 mm, *SD* 1 mm) and die three to seven months after infection. The monkey was therefore killed ninety-four days after infection; two female worms (measuring 24 and 15 mm) and three males, two alive (15 and 12 mm long) and one dead and encapsulated, were recovered from the subcutaneous connective tissues of the abdomen and thorax. The females had been fertilized and appeared normal histologically. They were small for their age (normal mean estimated at 35 mm; mean at 77 days 22 mm, *S.D.* 3 mm), although there is wide variation during this period of rapid growth. The living males were fully mature and of average size.

Dracunculiasis is still a major medical problem in parts of Africa and Asia and, although there have been several reports of successful treatment with niridazole[7-9] as a result of therapeutic trials in man, there has been no readily available laboratory model in which to study its mode of action.

Freezing of embryos for subsequent infection of cyclops should prove useful in the laboratory maintenance of the parasite, which has so long an incubation period and such a short period of patency in the laboratory host. It may also be applicable to other parasitic nematodes with free-living larvae.

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Parallel Evolution in Leaf Venation: an Alternative View of Angiosperm Origins

MELVILLE[1] advanced the hypothesis that leaf venation patterns of flowering plants provide a body of evidence suggesting the origin of angiosperms from the Permian *Glossopteris* and its allies, which flourished on the old continent of Gondwanaland. He shows that *Glossopteris*-like venation patterns are encountered in several living angiosperms, and emphasizes that most significant examples are found in regions which once constituted the Gondwanaland continent. His argument hinges on the extent to which these venation patterns can be said to resemble closely *Glossopteris* rather than other gymnosperm leaf types. This proposition can seriously be called in question.

Melville has also suggested[2-4] that the fructifications of some Glossopteridae, as reconstructed by Plumstead, have features in which they resemble the "Gonophyll"—the basic morphological unit of Melville's new theory of angiosperm flower structure. The proposition of the derivation of angiosperm leaf architecture from that of the *Glossopteris* leaf type is advanced independently of this, on its own merits, and can reasonably be judged on this basis.

Melville suggests that the Glossopteridean leaf is characterized by a "syndrome" of vein junction patterns (his Greek alphabet configurations are reproduced here in Fig. 1*E*), and that these are "associated together only in the Glossopteridae and in the more primitive leaf types of the angiosperms. Some of the characters occur in other groups, notably ferns and pteridosperms, but combined in different combinations to form other distinctive syndromes". Fig. 1 illustrates four leaf venation patterns selected from four different orders of fossil gymnosperms (Pteridospermales *sensu stricto*, Caytoniales, Cycadales and Bennettitales) each of which is believed (from evidence of the other parts of these plants) to be at least as remote from the Glossopteridae as they are from one another and from the angiosperms. All possess an impressively high proportion of vein junctions of the "glossopterid syndrome". While this venation syndrome is quite distinctive, and differs from that of most living angiosperms, it is matched closely in these several unrelated lines of gymnosperms, and indeed in several other groups of plants. On venation pattern alone there is no closer similarity between *Glossopteris*-like angiosperms and *Glossopteris* than between these and the four orders of gymnosperms illustrated.

It seems that one cautious conclusion can reasonably be drawn: the number of ways of developing a net-veined leaf from an initially dichotomizing system is limited. The "glossopterid syndrome" is part of a well-trodden evolutionary pathway, followed by several other groups. We clearly cannot use venation alone as a basis for reading angiosperm ancestry back into the Glossopteridae. As Melville notes, Foster has argued convincingly that some primitive living Ranales with an open (dichotomizing) venation pattern represent a primitive state within the angiosperms; so that if a single evolutionary origin is accepted for the angiosperms—a point on which we would agree with Melville—it seems likely that net venation developed within the group, probably some time in the Mesozoic, rather than having been already possessed by their putative Permian precursors. It is not suggested here that the rather widespread occurrence of the "glossopterid syndrome" rules out the possible derivation of the angiosperms from that group. It can only be argued that gross venation patterns present too little diversity, and are arrived at too readily by more than one evolutionary pathway, to form a valid basis for any phylogenetic conclusions.

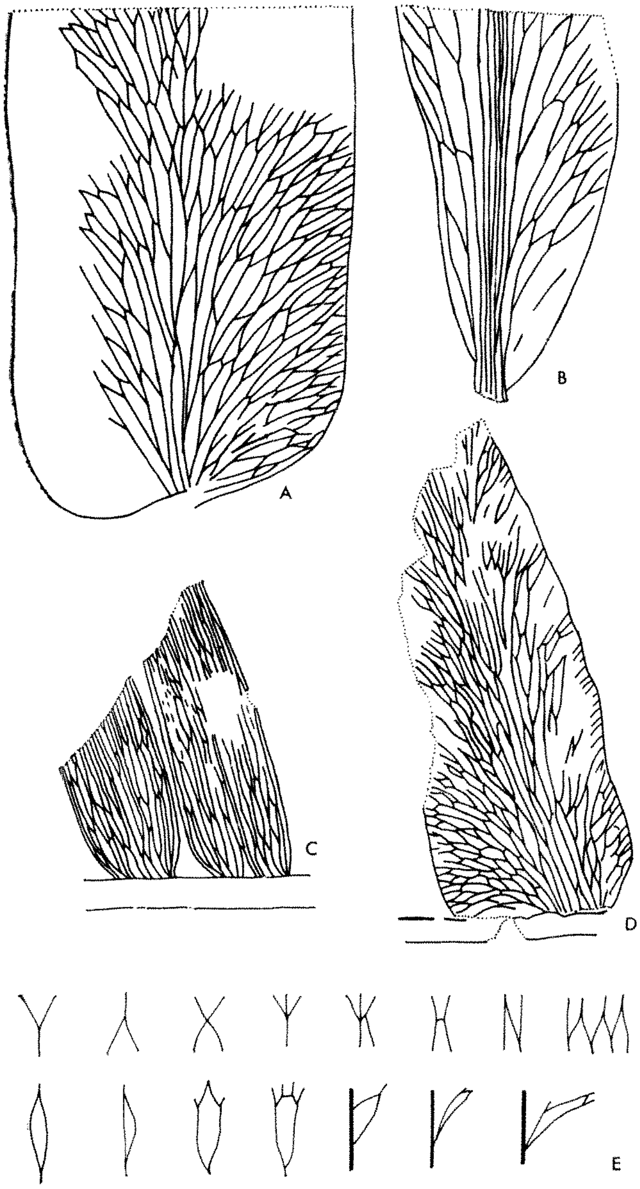

Fig. 1. Four different types of fossil gymnosperm leaf belonging to different orders, all with a significant proportion of vein junctions of the "Glossopterid syndrome" (*E*). *A*, Base of pinnule of *Linopteris obliqua* (Pteridospermales, Upper Carboniferous, × 4); *B*, base of leaflet of *Sagenopteris phillipsi* (Caytoniales, Middle Jurassic, × 4); *C*, bases of pinnae of *Ctenis kaneharai* (Cycadales, Middle Jurassic, × 1); *D*, *Dictyozamites hawelli* (Bennettitales, Middle Jurassic, × 4). (*A*, After Crookall[5]; *B* and *C*, after Harris[6]; and *D*, after Harris[7].)

Whether or not the reproductive structures of Glossopteridae are at all angiosperm-like is a closely related but none the less separate issue. But until more is known about this aspect it seems unfortunate to allow *Glossopteris* and its allies to hold the undivided attention of botanists as the principal candidate for the source of the angiosperms, for there is still woefully little evidence pointing to a solution to this problem.

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Reply to Criticism of Hypothesis of Leaf Venation and Angiosperm Origins

Alvin and Chaloner's criticism of my ideas about leaf venation patterns seems to be based chiefly on the distribution of vein junction types to the exclusion of associated vein island types. It is necessary to emphasize, therefore, that the glossopterid syndrome includes characters belonging to both of these categories. From the phylogenetic standpoint the important correlations are those between the complete syndrome and the major taxa: Angiospermae, Glossopteridae, Pteridospermae, Cycadales, Bennettitales and Caytoniales. Parts of the syndrome are lacking from all of these groups except the first two. What the critics do show is that similarities in the venation systems extend beyond that of open dichotomy—the *Ginkgo* and *Circaeaster* stage—as far as the *Gangamopteris* stage. We can agree to parallel evolution in these groups up to the *Gangamopteris* stage. Confining the discussion, for the moment, to leaf characters, there are some other features to be taken into account. The examples cited for the Pteridosperms, cycads and Bennettitales are all of leaflets of compound pinnate leaves, whereas the comparison was made between simple leaves in the Glossopteridae and Angiospermae. Compound pinnate leaves occur frequently in only about 20 per cent of angiosperm families and in many of these there is internal evidence of a derivation from simple leaves. One exception, the Quiinaceae, has already been commented on. The only other major exception is the Leguminosae in which the evidence is consistent with the persistence of compound leaves since the glossopterid phase of evolution.

The fourth example, *Sagenopteris* from the Caytoniales, is of a leaflet from a compound digitate leaf with a venation pattern representing an evolutionary stage between that of *Gangamopteris* and *Glossopteris*. The midrib is poorly developed, being a loose aggregation of parallel veins, and the principal vein islets are of the hydroid type which in the evolutionary sequence is one of the first to appear in *Gangamopteris*. Moreover, these vein islets run almost parallel with the midrib, whereas in the more advanced species of *Glossopteris* the principal vein islands along the midrib are of arcuate, angular or deltoid types and they make angles of about 70°–80° with a compact well formed midrib. The latter three types of vein island are the most advanced elements in the glossopterid syndrome. They must carry considerable weight in assessing probable angiosperm affinities with the Glossopteridae, for they occur in several angiosperm families in a similar relationship to the midrib of a simple (not a compound) leaf. Unless it can be shown that the more advanced types of vein island occur in the same kind of relationship in the other four orders of gymnosperms, the claim of Alvin and Chaloner that "on venation pattern alone there is no closer similarity between *Glossopteris*-like angiosperms and *Glossopteris*" than between these and the other major taxa cannot be maintained.

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Identification of Polychlorinated Biphenyls and DDT in Mixtures by Mass Spectrometry

Recently it was recognized that certain chlorinated aromatic compounds, particularly polychlorinated biphenyls (PCBs), are found in natural samples as pollutants[1,2]. PCBs interfere with the analysis for chlorinated pesticides because some of the PCB components from the technical mixture have identical retention times and respond to the electron capture detector[3,4].

Although combined gas chromatography–mass spectrometry for detection of PCBs from natural samples has been described[5], the use of mass spectrometry, particularly high resolution techniques, for the unambiguous identification of PCBs in the presence of chlorinated pesticides without previous separation[6] has not been reported.

We have recorded the mass spectra of a commercial sample of PCB ('Aroclor 1242') in different conditions and obtained high resolution measurements to confirm the molecular formulae of major components. The peaks arising from the individual major components of the mixture were as follows. $C_{12}H_8Cl_2$: found, 222·0000; calc., 222·0000. $C_{12}H_7Cl_3$: found, 225·9614; calc., 255·9610. $C_{12}H_6Cl_4$: found, 289·9220; calc., 289·9220. $C_{12}H_5Cl_5$: found, 323·8829; calc., 323·8830. (The experimental error is within ± 2 p.p.m. in all cases.) These can easily be distinguished from major peaks found in the most common chlorinated insecticides (Fig. 1 and refs. 6–13) by their position, peak pattern arising from the chlorine isotopes and particularly high resolution measurements. Furthermore, a useful spectrum of 'Aroclor 1242' can be obtained at low temperatures by direct introduction with the aid of a temperature controlled probe[14], and fractional sublimation from many chlorinated pesticides directly in the ion source seems possible (see Fig. 1).

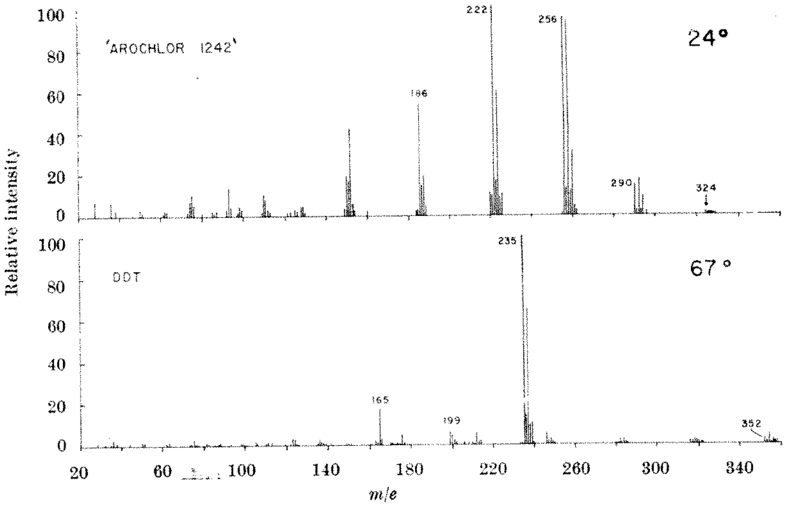

Fig. 1. 70 eV mass spectra (C.E.C. 21–110B instrument) of a 1 : 1 mixture of 'Arochlor 1242' and DDT at different sample temperatures. Isotope clusters at *m/e* 222, 256, 290 and 324 result from individual components of 'Aroclor'; the group of peaks at *m/e* 186 are mainly fragment ions from this substance.

Techniques for identification of trace quantities of PCBs and chlorinated insecticides in crude natural samples and their semiquantitative determination by mass spectrometry as well as the chemical characterization of major constituents of PCBs are being developed.

We thank D. J. Embree for recording the mass spectra.

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Book Reviews

LUMPS AND LOCALIZATION

Mind, Brain and Adaptation in the Nineteenth Century

Cerebral Localization and its Biological Context from Gall to Ferrier. By R. M. Young. Pp. xiv + 278. (Clarendon Press: Oxford; Oxford University Press: London, January 1970.) 65*s*.

THIS volume has attempted to record the development of ideas of cerebral localization in the nineteenth century, devoting major emphasis to Gall, Flourens, Magendie, Mueller, Bain, Broca, Spencer, Jackson, Carpenter, Fritsch and Hitzig, and finally Ferrier. It was clearly Gall who most intrigued the author, and we are indebted to him for a careful refutation of the common error of regarding the founder of phrenology purely as a charlatan. Young attributes to him the first empirical approach to the relationship between the functions of the brain and their localizations, and shows him as an able anatomist and a careful clinical observer of certain aspects of aphasia many years before Broca. Furthermore, he shows that while Gall stressed complex behaviours at the neglect of physiology, the experimental successes of later workers such as Ferrier led to the too ready assumption that the higher activities could all be compounded out of elementary motor and sensory components.

The debate on localization is one that will obviously go on for many years. Nearly all of the major approaches to it were developed during the nineteenth century. Young points out that no complete history of the problem has been available. His work, by thoroughly covering most of the important figures and problems, is therefore important. My only objection to his treatment is in certain omissions. This probably results from his admitted reliance for certain portions of his account on secondary sources. He has drawn very heavily on Henry Head, whose famous and undoubtedly influential discussions many of us would regard as biased, inaccurate and indeed contradictory because he himself ended up by supporting localizations which he had rejected violently earlier in the same volume.

This heavy reliance on secondary sources may account for the fact that, except for Broca and Fritsch and Hitzig, Young neglects almost entirely the contributions of the French and especially the Germans in this field, accepting the common bias in much of the literature in confining himself almost entirely after 1870 to English writings. He draws heavily on Hughlings Jackson, but neglects the fact that his influence outside England was not great, and that he was not well known in German circles until Sittig's translation of some of his writings many years later. By contrast, Meynert, whose comparative anatomical studies correlating structure and function, and whose division of fibre tracts of the brain into projectional, associational and commissural systems, so profoundly influenced German ideas on localization, is given only a few lines. Carl Wernicke, whose monograph of 1874, with its detailed discussion of localization theory and its special stress on anatomical connexions between cortical regions, dominated much of Continental thought in this field for the next half century, is mentioned only in a footnote.

This reliance on Head may also explain why Young probably underestimates the importance of the problem of aphasia in the history of theories of localization. He admits that Broca's discovery of a localized region for speech was the first functional localization which met with general acceptance by the scientific community. The acceptance of Wernicke's views was almost equally dramatic, because he was the first to provide and set up a plausible theoretical basis for the anatomical localization of language functions, from which he and his students claimed that it was possible to predict the existence of new forms of aphasia. Speech functions remained as a critical facet of most discussions of localization. Even the extreme holist could come to accept cortical sensory and motor systems by regarding them essentially as peripheral instrumentation, but he continued to balk at the idea of localizations for psychological functions, such as language. It was even possible for Sherrington to establish a reflex physiology of the spinal cord and brain stem and to be unaware that attempts at analysis of higher functions had, in fact, been more sophisticated than a simple phrenological model.

Young argues, in my view correctly, that a knowledge of the history and awareness of philosophical problems in this area is important for future advances. He has contributed importantly to this end, and one can only hope that he will look more fully in the future into the Continental contributions of the period 1870–90 to the controversy on ideas of localization.

NORMAN GESCHWIND

THE SOCIOLOGY OF FOOD

Man and Food

By Magnus Pyke. Pp. 256. (Weidenfeld and Nicolson: London, April 1970.) 35*s*.

INFORMED discussion about the food supply of a community is dominated by three equally important factors: how much of each of the known components of a diet is needed; how can the necessary quantity be supplied; how can we ensure a proper distribution so that everybody gets as much as is needed? Pyke deals with all three themes in a lively and humane manner. His book contains so much information and has so many merits that it deserves to be widely read. Nevertheless, it has some unevennesses. It is odd to find two pages devoted to the structural formulae of volatile oils and terpenes and one to the amino-acids, but no vitamin structures. Had he included the last, the meaning of niacin would have been clear. The word was invented because one group of manufacturers had difficulty persuading people (who probably did not need vitamin supplementation anyway) to buy nicotinic acid when other manufacturers were persuading them that nicotine in tobacco was harmful. Niacin is now acceptably defined as a blanket word for the acid and amide.

Two chapters on animal products explain, among other things, that the depletion of glycogen in an animal killed after a struggle diminishes the formation of lactic acid which inhibits bacterial growth. Hence the better keeping qualities of properly slaughtered meat and the poor keeping qualities of fish, which always die after a struggle. These chapters also explain the merits, and demerits, of cheese; the losses that are entailed in using animals to convert the protein in their food into the product that we eat; and the possibilities of using some at present wild animals. There is a brief chapter on cereals, vegetables and fruit.

Pyke argues convincingly that starvation and malnutrition usually have a political origin. It was the rapacity of landlords that forced the Irish to rely so completely on potatoes that the outbreak of blight in 1845 was devastating, and it is lack of money rather than inability to grow food that causes much present-day malnutrition. In the light of this conclusion it is a pity that he does not comment on the fact that FAO, in its "Provisional

Indicative World Plan for Agricultural Development", limits discussion to effective economic demand rather than need. He points out that gifts of food to countries with malnourishment often do more ultimate harm than good, and suggests that it would be better so to organize things that the people of these countries could earn more money and buy the food. It would seem to be still better so to organize things that they can grow the necessary food locally.

People with a mystical belief in "natural" foods are roundly trounced, and many examples are given of the indubitably beneficial effects of food processing. Examples are also given of potentially harmful processing undertaken for trivial or purely commercial reasons. Pyke recognizes that one cannot generalize about the activities of food manufacturers. They need watching, but the watchers should not become paranoid. The standards that are sometimes now set would have condemned rhubarb, and even perhaps the potato, had they operated when these plants were introduced into Europe.

An excellent final pair of chapters discuss population problems and the potentialities of synthetic foods, the extraction of leaf protein, and the cultivation of edible microorganisms on agricultural wastes and petroleum. Here, and at many other points in the book, Pyke stresses the need for greatly increased research; we know, for example, very little about the food needs of the elderly. He shows commendable scepticism of the simple explanations sometimes given for recent population increases and argues that they are not caused simply by medical progress but by many forms of technological innovation. A little technological change is welcome, but he sees the risk that too much may make life unbearable and produce plethora —not just of food, but of noise, crowding and pollution as well. N. W. PIRIE

TOOLS FOR PLANT BREEDERS

Induced Mutations in Plants

(Proceedings of a Symposium on the Nature, Induction and Utilization of Mutations in Plants, jointly organized by the IAEA and FAO, and held at Pullman, Washington, 14–18 July 1969. Proceedings Series.) Pp. 748. (International Atomic Energy Agency: Vienna; HMSO: London, 1969.) 517 schillings; 166*s* 8*d*; $20.

MUTATIONS occur spontaneously, but rarely. Radiation and mutagenic chemicals are tools with which the plant breeder may induce more variation for selection, but mutations are chance events and, unless understood and controlled, induced mutations as a weapon in the breeder's armoury might be compared with trying to hit the bullseye with a shotgun; one may be successful but the target has to be rebuilt. This book constitutes the published papers of a symposium intended to review our understanding of the use of induced mutations in plants.

The symposium was divided into seven sections. Section A consists of nine papers on the nature of mutations, genetics and biochemical pathways; section B, fifteen papers on the action of physical and chemical mutagens; section C, four papers on the induction of mutations and breeding methods in vegetatively propagated species; section D, fifteen papers on induced mutations and breeding methods in seed-propagated species; section E, seven papers on the utilization of mutations for general and evolutionary studies; section F, three papers on the use of chromosome aberrations in breeding; and section G, three papers on new induced mutant varieties. Of the fifty-eight papers, fifty-two are in English and all are preceded by brief summaries in English. Excepting D and E, the first paper in each section is chiefly devoted to a comprehensive review (with extensive bibliography) of the topic under discussion.

The papers in section A provide evidence for increasing sophistication of techniques. R. D. Brock's paper on "Increasing the Specificity of Mutation", for example, describes experiments with ***Escherichia coli*** which indicate that certain mutagens will mutate an active gene more readily than when it is inactive. Also, by using the regulator protein of the lactose operon, made mutagenic by incorporating ^{35}S and ^{3}H amino-acids, he opens up the prospect of inducing mutations at particular loci. If similar systems are operative in higher plants, refined control of the mutagenic processes may be possible.

References to the successful use of induced mutations are many. These include the use of neutrons and X-rays to induce *Verticillium* resistant strains of peppermint, by M. J. Murray (section C); the use of X-ray induced translocations to produce the first commercial F_1 hybrid barley, by R. T. Ramage (section D); the ability to increase the yield of barley some 10 per cent above that of the mother variety, described by members of the Max Planck Institute (section D); not to mention the scores of released crop varieties described and listed in section G.

In section E, along with papers on the "Tetrasomic Heredity of Induced Mutations in *Arabidopsis thaliana*" and the "Nature of Chromosome Deletions", R. F. Stettler and co-workers discuss the interesting possibility of producing homozygous forest trees by induced haploid parthenogenesis. The diversity of the papers in section B supports the view of M. S. Swaminathan that breeders should continue to use a wide range of mutagens.

These proceedings may be of particular interest to British plant breeders who, in a list of one hundred and one participants from twenty-five countries, are sadly noticeable by their absence. G. R. MACKAY

ESSENTIAL NUTRIENTS

Trace Elements in Agriculture

By Vincent Sauchelli. Pp. viii+248. (Van Nostrand Reinhold: New York and London, January 1970.) 140*s*.

VINCENT SAUCHELLI died in autumn 1969 after a long life in agricultural science, latterly in association with the American fertilizer industry. This is his last book; it was written as a survey for the general reader and student and as background information for advisers, teachers and fertilizer manufacturers who meet trace element problems in their daily work. It succeeds in these aims.

The first five chapters are introductory. They describe the historical background to trace element research, and biological and physico-chemical relationships of crops and soils involving trace elements. This information is simply but accurately presented and will be useful to both students and to those who need to refresh their memories.

Six chapters follow on the trace elements or micronutrients which are well recognized as being essential to plants—iron, manganese, boron, zinc, molybdenum and copper. These chapters have a general pattern. Sources of trace elements in soils, the effects on plants of deficiencies and surpluses, and the effects of each element on animal health and nutrition are described. The methods used to correct deficiencies are discussed well; the tables setting out recommendations for different crops and soils in parts of the United States are particularly valuable features of some of the chapters. There is a chapter on chlorine, the latest element to be accepted as essential for plants, but which is much more important in animal nutrition and health.

Sodium seems to be essential for a few plants and can substitute for potassium in others. There is a chapter on sodium, but it gives very little information about the importance of this element in animal nutrition. But sodium is hardly a trace element, either for plants or animals, and the account is out of place in this book.

Following chapters deal with selenium, cobalt, iodine and fluorine; all of these are essential to animals and cobalt is essential for leguminous plants. There are brief accounts of the possible effects on plants and animals of nickel, lithium, vanadium, silicon and aluminium.

The text is concluded with a brief but wise discussion on correcting trace element deficiencies, and the possibilities of incorporating them into mixed fertilizers supplying major nutrients. Sauchelli did not recommend "shotgun" applications where deficiencies had not been diagnosed. There is a list of commercial American producers of trace elements and methods of analysis are briefly described.

It is a relatively short book, but sufficient for agricultural students and it is recommended to them. Advisers and research workers with trace element problems will have use for the accounts of deficiencies and methods of correcting them. Further information can be got from the numerous references. Sauchelli read widely and European literature is well represented in the bibliographies (this is not always so in American books on agricultural science). The book is well printed, but the black-and-white illustrations of deficient plants are little help (there are no colour plates). It is very expensive and the price will prohibit purchase by many who would find it useful.

G. W. COOKE

COMPARTMENTAL ANALYSIS

Multicompartment Models for Biological Systems

By G. L. Atkins. (Methuen's Monographs on Biological Subjects.) Pp. xiii + 153. (Methuen: London, November 1969.) 35*s*.

WHEN a substance such as a radioactive tracer is introduced into a biological system, its concentration in any particular part of the system may first rise and thereafter declines asymptotically to a steady level, which may be zero. Such a curve can usually be fitted empirically by super-position of a few exponential components. This, as it happens, is exactly the behaviour of a tracer in a system consisting of a number of well mixed pools or compartments which interchange material with each other at a constant rate; the number of exponential components is one fewer than the number of compartments (which may possibly include the external environment). Compartmental analysis consists in the derivation of concentration curves for various models and conversely in the devising of models to fit experimental concentration curves. There is a temptation to identify the compartments arising from the analysis with the recognizable anatomical divisions of the biological system. This identification, however, is open to the objection that there is very great latitude in fitting functions to a set of experimental points subject to even moderate error. Given *a priori* knowledge of the number of compartments there is very great imprecision in determining the size and exchange rate of any one of them. Moreover, there is seldom any guarantee that an anatomical division behaves as a single homogeneous compartment, and even if one component more than the minimum required to achieve a satisfactory fit is introduced, the range of possible combinations of functions is further enormously increased.

These considerations, which a little experiment will easily verify, might well cause a reader to doubt whether the process of compartmental analysis is of any scientific value, and if he approached Dr Atkins's book in this frame of mind, he would find little to dispel his scepticism. This is the weakness of the book; it deals too briefly with the very matter for which there is the greatest need, the appraisal of the validity of the solutions obtained. The chapter on treatment of experimental data and curve fitting is decidedly thin. In fairness to the author, however, a really adequate treatment would need to be quite elaborate, possibly too elaborate for the audience he has in mind.

The book does not attempt a formal exposition of the subject, such as is already available in essence in the usual texts on differential equations; it is rather a survey of the techniques of compartmental analysis and of their fields of application. This is supported by a fairly extensive and well chosen bibliography. A non-rigorous mathematical treatment is introduced as required, with a brief treatment of the differential operator, D, and of the Laplace transform given as an appendix. This approach will probably be congenial to a reader with some mathematical equipment who wishes to comprehend the general mathematical strategy without actually becoming expert himself. Such a reader will be glad of the chapter on simulation techniques in which methods of obtaining analogue representatives are described. The use of digital computers is only touched on, but sufficient references are given to lead the reader towards the help he may need.

E. W. EMERY

TISSUE INCOMPATIBILITY

Immunogenetics of Tissue Transplantation

By A. Lengerová. (North-Holland Research Monographs Frontiers of Biology, Volume 16.) Pp. xv + 270. (North-Holland: Amsterdam and London, 1969.) Hfl. 45; 126*s*.

As Dr Alena Lengerová explains in her welcome book, immunogenetics used to be thought of largely as the application of immunological techniques to the solution of genetical problems. This is no longer so, for the study of tissue and organ transplantation (with its extension into the fields of auto-immunity and tumour immunology) is now a science in its own right in which an understanding of the genetical control and inheritance of cellular antigens is of paramount importance. It is entirely appropriate that immunogenetics should provide us with such a fine example of hybrid vigour: here, two branches of science converge and interact in the liveliest manner, and we need hardly be surprised that this interaction has been so outstandingly fruitful.

The book arose from a lecture course given by this Czech scientist in 1968 at the University of Indiana and it was never intended to embrace the whole field; it does not, for example, include, except in passing, the genetics of human blood groups or of allotypic determinants. Instead, the author sticks closely to her brief of analysing the immunogenetics of the cellular antigens responsible for the induction of tissue incompatibility, and this she does superbly well. It seems nevertheless surprising that she has not devoted a chapter to the genetics of human transplantation antigens: although (and perhaps just because) this new branch of immunogenetics is still in a relatively rudimentary state it would have been most helpful to the general reader to have had the benefit of Dr Lengerová's guidance and critical appraisal.

The H-2 locus of the mouse—that much studied histocompatibility locus controlling the appearance of the strongest and therefore, by definition, the most important transplantation antigens—is naturally given pride of place. Dr Lengerová does not scorn a historical account when required, and it is good to be reminded of how much we owe to the work of the late P. A. Gorer and to G. D. Snell and their colleagues. While never oversimplifying, the author frequently finds it necessary (and understandably so) to get down to first principles, and it is this quality of lucid exposition that will make this book a valuable reference text, not only for immunologists but also for students of genetics, biology and kindred subjects. When first coming to grips with immunology, students are frequently astounded by the relative lack of hard data and the numerous rival theories; in immunogenetics, too, there are glaring gaps in our knowledge. For example, we still do not know with any degree of certainty just how the H–2 system is organized and how it operates. Is it one locus with multiple alleles and a single as opposed to several mutational sites per allele, or is it a cluster of closely linked loci, each with a number of alleles? Or are the manifold specificities generally ascribed to the H–2 locus the result of some kind of interaction between the gene product of the H–2 locus and those of other loci? Dr Lengerová discusses these various possibilities in some detail and she presents the evidence from which all at present derive some measure of support.

Another topic dealt with in depth is that of the male-linked histocompatibility antigen, namely, the antigen responsible in many inbred strains of mice for the rejection of male skin grafts by female recipients. In the literature this is generally referred to as an antigen controlled by a gene carried on the *Y* chromosome, and certainly the experiments of F. Celada and W. J. Welshons and others provide strong (if not conclusive) evidence in favour of this hypothesis and against its rival, which would explain the presence or absence of the male-specific antigen in terms of the absence or presence of the second *X* chromosome. The situation is nevertheless complicated by the demonstration that neonatal orchiectomy in some measure inhibits the phenotypic expression of this antigen, namely, that the presence of male hormones is required early in life if the antigen is to be fully developed (orchiectomy 10 days after birth having no effect).

Dr Lengerová devotes other chapters to topics such as tumour-specific antigens, the thymus-leukaemia (TL) antigenic system of the mouse, the development of histocompatibility antigens, the concept of "strong" and "weak" antigenic differences, antigenic mutation, estimation of the number of H-loci, the biological role of H-antigens, the phenomenon of allogeneic inhibition, and the peculiar status of the foetus that permits it to escape immunological attack by the mother. The book as a whole is written in impeccable and lucid English, and the reader has cause to be grateful to the author for dealing with a difficult subject with such clarity and authority. The publisher has produced the book very competently: my only criticism here is directed at the very inadequate index, which is almost entirely based on authors rather than subjects—and the price.

Leslie Brent

REPRODUCTIVE ENDOCRINOLOGY

Immunoassay of Gonadotrophins

Transactions of the First Symposium held in Stockholm, September 23–25, 1969. Edited by E. Diczfalusy, assisted by A. Diczfalusy. (Karolinska Symposia on Research Methods in Reproductive Endocrinology.) Pp. 382. (Reproductive Endocrinology Research Unit: Stockholm, 1969.) n.p.

This book is comprised of papers describing up-to-date methods in the field of immunoassay. The volume, a result of active collaboration between the Karolinska Institute and the World Health Organization, has been published within four months of the symposium and is distributed to the libraries of every medical school in the world "in order to stimulate new scientists to enter the field", and to form a basis for the development of new methods of assay in reproductive endocrinology.

The chemistry of gonadotrophins in relation to their antigenic properties is described. As well as some chemical differences between follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotrophin (HCG), each hormone seems to have antigenic groups which are distinct from those parts of the molecule which are essential for biological activity. There are immunological similarities between some of the human anterior pituitary trophins, which makes it difficult to produce antisera useful in specific assays; the immunological cross-reaction between HCG and LH is also well established. The preparation of radioiodinated human LH and FSH is described, as also are the inaccuracies due to iodination damage to the hormone and the effects on precision and sensitivity caused by the immunoreactivity of the labelled preparation used. It is essential that a labelled hormone to be used as tracer should be of high specific radioactivity and that both the hormone to be measured and the standard should displace the tracer in an identical manner.

A variety of methods have been used for the determination of gonadotrophins in both plasma and urine. Agglutination inhibition reactions (using erythrocytes or latex particles for sensitization) and complement fixation tests have been used as a guide in both normal and abnormal pregnancy. The use of chromatoelectrophoresis or antibody coated disks and tubes for the separation of antibody-bound and free labelled hormone in the assay of LH and FSH in clinical application is discussed. Immunosorbents with high stability, high capacity to bind antigen and little tendency to non-specific adsorption of proteins and polypeptides, can be coupled covalently to insoluble polymers to form stable conjugates with retention of immunological binding capacity. The double antibody technique is very simple to use—its disadvantages are the difficulty in assessing damage variability from serum to serum, variability with different lots of second antibody and the necessity of assessing the specificity of the assay with each amount of carrier protein used.

This is the first time that various methods of immunoassay of gonadotrophins in body fluids have been brought together—the book will serve as a stimulus to further work in a relatively new field. As yet no single technique satisfies all the criteria needed for an assay for routine clinical work and reproduction research—namely, one which uses small samples of fluid, is simple, specific and reproducible. In general, similar patterns of LH and FSH in serum during the menstrual cycle have been obtained from different laboratories, but there is a significant variation in quantitation in different parts of the cycle (and especially of the mid-cycle peak of LH) probably as a result of variations in methodology. It is essential that reliable standards are established and, in particular, a separate standard for urine and pituitary (or serum) gonadotrophins must be used, because gonadotrophins in urine seem to have lost some of their immunological potency—possibly as a result of the metabolism of the hormones—leading to a variation in the results when assayed against a pituitary standard.

May Reed

MUSCLE MOVEMENT

Muscles and Movements

A Basis for Human Kinesiology. By M. A. MacConaill and J. V. Basmajian. Pp. viii + 325. (Baltimore, Maryland: Williams and Wilkins, 1969. Distributed in the UK by Livingstone: Edinburgh.) 90*s*.

The ultrastructure and electrophysiology of muscles having held the stage in recent years, it should now be a pleasure to read a book in which the functional need for these structures in man is examined. The pleasure is unfortunately mixed with some concern and irritation. The book falls fairly clearly into two parts. The first is an introduction to the theory of kinesiology based on the geometry and mechanics of bone and joint movement. It is presented as a statement of fact and with such authority that little room for doubt, discussion or even bibliographic support seems necessary to the authors. No hesitation is shown in coining new words or substituting unfamiliar for familiar terms. Swing, slide and spin provide a pretty example of alliteration but become confusing in practice, and the use of the term "myone" for motor unit has little advantage. An attempt has been made to simplify the mathematics of the subject, but this is marred in more than one place by inaccuracies in the text and the use of diagrams which, in spite of the authors' exhortation, do not clearly indicate all the forces involved in a mechanical problem. Physiology, apart from a possibly misleading reference to the lengthening of a muscle fibre "by simple relaxation", is intentionally ignored and as a result the impression is gained that the design of muscles is determined only by their geometry.

The remainder of the book is a useful summary of contributions made to the study of muscle action *in vivo* which have not yet been incorporated into standard texts. Many of these contributions have been made by the authors themselves and adequate reference is given to others working in this field. Old and new theories are discussed in the light of electromyographic findings and admission is made where these have yet to provide a complete answer.

In a final short section on "applications and problems", acknowledgment of the variability of human nature and physique must provide little to convince many of the readers at whom this book is aimed that their labour has been worthwhile. Reference to the book in the teaching of human morphology will, however, help in the selection of detail of practical value and give rhyme and reason to the variety of musculoskeletal design in man.

E. C. B. HALL-CRAGGS

CRUSTACEAN TAXONOMY

Treatise on Invertebrate Paleontology

Part R: Arthropoda 4. Directed and edited by Raymond C. Moore. (Prepared under Sponsorship of the Geological Society of America.) Vol. 1: Pp. xxxvi + 398. Vol. 2: Pp. ii + 399–651. (Geological Society of America: Boulder, Colorado; University of Kansas: Lawrence, 1969.) $19 the two volumes.

THESE long-awaited and most welcome volumes of the *Treatise on Invertebrate Paleontology* are noteworthy for the large panel of distinguished palaeontologists and neontologists who have contributed to them. The panel is composed of the seventeen authors and a large number of other specialists who have advised or assisted. The present two volumes are devoted to Crustacea (except Ostracoda, which are dealt with in part Q which has already been published) and Myriapoda; a third volume on the Hexapoda is in course of preparation.

As in the other parts of the treatise the essence of the volumes is a presentation of the current views of the individual authors regarding the classification of the Crustacea and Myriapoda, with a concise diagnosis for each taxon down to generic level. For this reason alone they become definitive works and an essential source of reference for those working on crustacean taxonomy. But, more than this, a large part of these particular volumes is devoted to accounts of the morphology, ontogeny, mode of life and stratigraphy of the classes, which with good bibliographies make them invaluable source books for students of zoology as well as palaeontology.

Although they form part of a palaeontological treatise, these volumes acknowledge the fact that there has been a major difference of views between palaeontologists and neontologists regarding the evolution of the Crustacea, and each camp has its own version of a natural classification. Thus it is of particular interest that two of the most stimulating contributions to the volumes, both by Sidnie Manton, are devoted to an "Introduction to Classification of Arthropoda" and to the "Evolution and Affinities of Onychophora, Myriapoda, Hexapoda and Crustacea". Similarly, the valuable introduction to the Decapoda by M. F. Glaessner includes a review of their phylogeny.

As might be expected in a compilation of this kind, there are points on which specialists in the various fields might wish to differ with the contributors. This does not, however, detract from the overall value of an important work in a hitherto much neglected field of palaeontological studies. All of the contributors, and in particular the director and editor R. C. Moore (who has in this as in other parts of the treatise taken under his wing the inevitable "orphan" groups which no one wants to own, plus a lengthy "General Features of Crustacea" section in conjunction with L. McCormick), are to be congratulated on the results of their labours.

Palaeontologists, already familiar with the format, excellent standard of production and ample illustration of other parts of the treatise, will need no other comment than that these volumes are among the best so far published (though, it must be added, they are also among the most expensive). Zoologists who will be less familiar with the treatise are urged not to neglect what at first sight of the title might seem to be, for them, barren territory.

H. W. BALL

CIRCULATION STUDIES

General Climatology, 2

Edited by H. Flohn. (World Survey of Climatology, Vol. 2.) Pp. xii + 266. (Elsevier: Amsterdam, London and New York, 1969.) 225*s*.

THIS is one of a series of fifteen volumes, the first three of which, with the title *General Climatology*, are to consist of chapters written by well known specialists. This volume, containing five of these essays, is the first of the three to be published.

In the opening chapter on mechanisms of the general circulation of the troposphere, H. Riehl begins by giving a brief review of the classical model leading to an account of model experiments. He then discusses critically analytical solutions of the high-tropospheric wind field, the mechanism of momentum exchange and of heat and moisture exchange, the kinetic energy generation and maintenance and the general circulation in wave coordinates, concluding with the remark that "understanding of the general circulation will be advanced by choosing the coordinate system most appropriate to the type of circulation studied".

E. L. Deacon, under the title "Physical Processes near the Surface of the Earth", surveys work on microclimates over level uniform terrain and extensive water surfaces. He examines in detail the whole range of surface-atmosphere interactions and includes accounts of the different kinds of fog and of turbulent diffusion of air-borne material.

R. Geiger extends these chiefly microclimatic studies to sloping terrain in a chapter on topoclimates, topoclimatology being defined as the subdivision of general climatology dealing with relationships between land forms and local climates. Beginning with the astronomical theory and examining the effects of screening of the horizon and of clouds, he describes the construction of radiation maps and then the climate of slopes generally. Succeeding sections deal with the climate of valleys, microclimates in mountainous regions, including the climate of caves, and the difficulties of the mapping of topoclimates. He forecasts that "the use of topoclimatic maps will become quite usual in the foreseeable future in the same way as we now use geological maps showing the subsoil or the ground surface for planning purposes".

The editor of this volume, H. Flohn, contributes a chapter on local wind systems, beginning with accounts of examples, followed by the theory, of simple thermally driven circulations, land and sea breezes. Cases of more complicated systems of mountain winds with a diurnal component are then described and analysed theoretically. Lastly, he examines effects on the wind field which are mechanically produced by mountains or escarpments, for example, the generation of the föhn, the bora, the mistral and lee-wave systems.

In the final chapter on climatic fluctuations, H. H. Lamb, after a short summary of the evidence of changes, ice ages and warm epochs, in the remote past, deals in more detail with the main climatic phases of post-glacial time, the past 10,000 years, concluding this section on climatic history with an analysis of observations in the era of meteorological instruments. An account is then given of circulation studies, the preparation of maps of prevailing wind and pressure patterns in different years and epochs, which may reveal the mechanism of climatic changes. The results of the analysis of the author's own series of maps and indices for the period from 1750 and his current work on the extension of the circulation analysis to years before 1750 are described. The chapter ends with a short summary of the causes and course of climatic variation.

This is a very good book which will be valuable to the meteorologist as well as to the climatologist.

JAMES PATON

BOUNDARY LAYERS FOR ENGINEERS

Boundary Layers of Flow and Temperature

By A. Walz. Edited and translated from the German by Hans J. Oser. Pp. xv + 297. (MIT Press: Cambridge, Massachusetts, and London, January 1970.) 164*s*.

BOUNDARY-LAYER theory has formed a major part of fluid dynamics since Prandtl's famous paper was published in 1904. Its first achievement was to resolve the conflict between the hydromechanics of the mathematicians and the hydraulics of the engineers; the former subject was elegant, extensive and founded on a plausible idealization of the behaviour of fluids; the latter was crude, empirical and fragmentary, but much more reliable as a predictor of the actual behaviour of real fluids. Prandtl showed that the small viscosity of these fluids, though negligible in its direct effect over a large part of the domain of flow, could have substantial indirect effects, emanating from the thin layer of fluid adjacent to the solid boundaries of the domain. The subsequent detailed investigations of particular boundary-layer flows completely clarified the qualitative phenomena which arise.

Boundary-layer theory has influenced engineering practice more through the understanding which it promotes than by providing quantitative methods of design; for the differential equations were too difficult to solve exactly until computer methods were devised; and the laws of turbulent transfer, which dominate most practical flows, are only now being discovered. In the past few years, however, fast computational procedures of the finite-difference variety have become available; and satisfactory mathematical models of turbulence have been developed for use with them. Boundary-layer theory is at last ready for use by engineers.

The demonstration of the usability of boundary-layer theory is one of the motives of Professor Walz's book. The material and methods of the book spring, however, from the pre-computer years during and after the Second World War. Thus, from the mathematical point of view, its procedures are approximate rather than rigorous; and the properties of turbulence are incorporated by way of empirically based correlations of the behaviour of complete boundary layers. Though the use of the computer is now advocated for the execution of the calculation methods, the full potentialities of the computer are not exploited.

Although the book is therefore likely to have come rather too late to influence engineering practice widely, its high quality as a piece of exposition deserves recognition. The treatment is scholarly and, granted the basic limitation just mentioned, it is both comprehensive in coverage and fair in evaluation. The author has taken much trouble to ensure that his methods of computation can be understood and practically applied. Engineers will find his methods to be fast, fairly easy to understand, and, within their rather narrow limits of validity, sufficiently accurate.

A few critical remarks should be made, however. The translation from the German is excellent in general, but marred by some inelegancies of construction or diction; thus the choice of the word "clot" for a coherent body of fluid in turbulent motion has led to some comic sentences. More serious is the disregard of the possibility that the solutions of the integral equations, which are Walz's preferred medium of computation, may not be unique; yet this often occurs when the velocity profile is "weighted", in the formation of these equations, by some function of the velocity, u; and it prevents the computation process from being completed. Because "weighting" functions of the cross-stream distance y are free from this disability, the author's preference for u-functions over y-functions may mislead his readers; it can be explained only by the fact that the author has not yet tried to apply his method to problems in which a maximum is present in the $u(y)$ profile, giving rise to two values of y for one of u. Again, Walz recommends von Karman's modifications of the Prandtl–Taylor relation between friction and heat transfer, without mentioning that it brings very little improvement, and is inferior to the more recent formulae of Deissler and others.

In spite of these blemishes, the book remains a valuable contribution to the boundary-layer literature. It was well worth translating.

D. B. SPALDING

PARTICLE TRANSFER

Introduction to the General Theory of Particle Transfer

By V. Kourganoff. (Documents on Modern Physics.) Pp. xiii + 216. (Gordon and Breach: New York and London, January 1970.) $16·80; 140*s*.

THIS book is an attempt to present a graduate student at the beginning of his course with a simple account of the way in which problems in particle transfer can be formulated in terms of differential equations. It provides a meticulous account of the way in which a field of moving particles interacts with a material medium. The mathematical analysis has deliberately been kept simple so as to focus attention on the physical concepts and to allow those who intend to specialize in, say, transport of neutrons to see something of the relationship of their chosen field with that of radiative transfer.

A book of this kind has been needed for some time, but it is not clear that this one properly fills the gap. The level of knowledge assumed is often extremely low—it should hardly be necessary to have to give a proof of Gauss's theorem to graduates—and a good deal of space is occupied by recording minor steps in the argument which the reader might be expected to supply for himself. It is also disappointing that the physics of the microscopic interactions of neutrons and photons with matter is not treated more comprehensively. The mathematical shape of the source and sink terms which determine the complexity of transfer theory depends on these interactions. This book will only give the student a very limited understanding of the relationship of the physics to the more mathematical problems treated in other texts. It is, moreover, extremely expensive for its size, the price being one which few students are likely to want to pay.

I. P. GRANT

POCKET MATHEMATICS

The Coordinate Method

By I. M. Gelfand, E. G. Glagoleva and A. A. Kirillov. (Primer 1.) Pp. xi + 68. 75*s*.

Functions and Graphs

By I. M. Gelfand, E. G. Glagoleva and E. E. Schnol. (Primer 2.) Pp. vii + 102. 75*s*.

Sequences and Combinatorial Problems

By S. I. Gelfand, M. L. Gerver, A. A. Kirillov, N. N. Konstantinov and A. G. Kushnirenko. (Workbook 1.) Pp. vii + 85. 75*s*.

Learn Limits Through Problems

By S. I. Gelfand, M. L. Gerver, A. A. Kirillov, N. N. Konstantinov and A. G. Kushnirenko. (Workbook 2.) Pp. vii + 70. 65*s*.

Mathematical Problems

An Anthology. By E. B. Dynkin, A. A. Molchanov, A. L. Rozental and A. K. Tolpygo. (Workbook 3.) Pp. vii + 69. 75*s*.

Limits and Continuity

By P. P. Korovkin. (Course 1.) Pp. vii + 125. 85*s*.

Differentiation

By P. P. Korovkin. (Course 2.) Pp. vii + 86. 75*s*.

(Pocket Mathematical Library.) Revised English edition; translated and edited by Richard A. Silverman. (Gordon and Breach: New York, London and Paris, May 1969.)

THERE are many texts dealing with school mathematics and introductory university mathematics; but none (in English) with quite the flavour of the present series of seven small books, translated from the Russian. The series consists of two primers, three workbooks and two courses. The primers are concerned with coordinates, functions and graphs, while the workbooks emphasize problems, especially involving sequences, combinatorics and limits. The two course books deal with limits, continuity and differentiation. All books are well written, with illustrations and worked examples, and every enthusiastic mathematics sixth-former may read them for pleasure and profit, and especially in order to be introduced to some of the problem solving encountered at this level in the Soviet Union.

One of the most interesting of the volumes is that entitled *Sequences and Combinatorial Problems*. Here, eighty-nine sample problems are listed and detailed solutions are given. There follows, without solution, a set of thirty-seven test problems. There are some unusual problems relating to arithmetic and geometric progressions, but the combinatorial problems are unfortunately confined to problems of counting. The latter, however, may be due to the difficulty of constructing elementary structural problems of a combinatorial character with simple solutions. The volume entitled *Mathematical Problems: An Anthology* is a gem. It consists of 143 problems, of many different types, with hints and answers, and a set of thirty-five supplementary problems. Some of the problems are tantalizing in their simplicity of statement. It may be of interest to quote two examples:

> Problem 1.—A million lines are drawn in the plane such that no two lines are parallel and at least one other line passes through the point of intersection of each pair of lines. Prove that all the lines are concurrent.
>
> Problem 2.—Three rugs of arbitrary shape, each with an area of 3 square yards, are spread on the floor of a room whose area is 6 square yards. Prove that two rugs overlap and share an area of at least one square yard.

In conclusion, it is worth recording a thought engendered by a reading of the books under consideration. It is clear that in elementary mathematics, as expounded in the Soviet Union, emphasis is still placed overwhelmingly on the classical concepts and sense of values (such as manipulation, but not for its own sake, and problem formulation and solution). It is still respectable to deal with counting problems involving permutations and combinations, and to ask and answer questions which involve an appeal to Euclidean geometry. In fact the biggest single surprise on reading the books is that of finding the degree of emphasis still placed on geometrical thinking. This retention of the "classical" mathematics rather than the wholesale acceptance of the so-called "new" mathematics is a sobering thought, which might well be followed by mathematicians in the western world, especially those concerned with the revision of school mathematics syllabuses.

L. S. GODDARD

QUANTUM MECHANICS

Basic Quantum Mechanics

By J. M. Cassels. (European Physics Series.) Pp. viii + 184. (McGraw-Hill: London and New York, 1970.) 62*s* boards; 42*s* paper.

IT is clear from the preface to this book that Professor Cassels endured all the usual agonies in learning quantum mechanics but was finally so impressed by the elegance and logic of Dirac's presentation that he is determined that his students shall not suffer the same fate. His solution is to present the subject as a set of concepts and rules followed by the logical consequences one may deduce for simple systems, but working throughout at a more elementary level than does Dirac. This seems almost certain to be unpopular with the older generation of physicists accustomed to the concept of the historical approach, but I must come down on the side of the author. If quantum mechanics always seems a difficult subject to teach to physics students, it is surely because the physical picture exists not in terms of classical models but rather in the operator structure, so that the natural starting point must be with the language which works rather than with that which does not.

In general terms the book seems to achieve what was intended, the choice of topics being particularly good, but there remain a number of shortcomings. First, a rather high degree of physical background seems to be required for what is supposed to be a basic text. The reader must be presumed to have been exposed to a fair amount of "the breakdown of classical physics", for no introduction of this type is incorporated. This seems particularly unfortunate, because such experience, insensitively given, could negate the whole concept of this style of presentation. Second, insufficient emphasis seems to have been given to the distinction between the basic quantum techniques and those approximations used in applications, so that there is a danger that students may confuse the two concepts. It is pleasing, however, to see such useful tools presented so early in such a basic course. Finally, there are no problems on which the student may test his comprehension. Experience in teaching this subject convinces me that no student really understands the concepts until he has "got his hands dirty" himself.

Considered as a whole, this is a good book for a first course in non-relativistic quantum mechanics, but it should be adopted only by an experienced teacher who is convinced of the superiority of this method of presentation.

K. J. BARNES

Obituaries

Sir Boris Uvarov

The death of Sir Boris Uvarov, KCMG, DSc, FRS, on March 18, aged 82, has deprived the science of entomology of one of its most eminent men. Best known as a taxonomist—for his description of some 900 new species and 218 new genera, and an ecologist—for his work on the phase theory of locusts, insects and climate, and arid zone entomology, he will also be remembered as the chief founder of international cooperation in locust research and control, and as a pioneer in the biogeographical studies of locusts, forming the scientific basis for the foundation of many international organizations.

Boris Petrovitch Uvarov was born in 1888 at Uralsk in Russia and obtained a first class degree in biology in 1910 at the University of St Petersburg. There followed a period of work in several provincial departments of agriculture and in 1915 at the age of 27 he was made director of the Tiflis Bureau of Plant Protection. He was already an authority on the Orthoptera of Central Asia and had become interested in locusts and their control; in 1919 he was appointed keeper of entomology and zoology in the State Museum and reader in entomology at the University of Georgia, and in 1921 published his famous paper propounding the phase theory of locusts. He was always punctilious in sharing the discovery of locust phases with Fauré, the South African entomologist, but Uvarov's name is universally associated with the theory, not only because he went on to develop it further but because of the practical use he made of it.

The practical opportunity came when he joined the Imperial Bureau of Entomology (now the Commonwealth Institute of Entomology) in London, where he remained from 1920 to 1945; the British government was concerned about locust invasions of the African colonies and Uvarov was the obvious man to consult. He suggested a programme of extensive field surveys in Africa to map the seasonal distribution of locusts, with ecological and biogeographical analysis in London. The results of this work, pursued over several years and interpreted in the light of the phase theory, revealed permanent outbreak areas for both the migratory and the red locust. Uvarov and others had also pointed out that cooperation between the affected countries was utterly essential for locust control, and a series of international conferences was held to promote this view, leading to proposals for international organizations in Africa to control the African migratory and red locusts in their outbreak areas. Progress was interrupted by the 1939–45 war, when the protection of strategic food supplies became vital; Uvarov helped to create the Middle East Anti-Locust Unit on a para-military basis to carry out control campaigns in many countries.

After the war, because of the threat of locusts to overseas agriculture, the Colonial Office set up the Anti-Locust Research Centre as an independent research institute, with Uvarov as director. Uvarov here continued his untiring effort against locusts; the basis of the Centre's work was then, and still remains, the view that efficient locust control is possible only with a background knowledge of the biology, biogeography and behaviour of the species.

The demands of administration had not diminished Uvarov's other interests; he was a world authority on the taxonomy, systematics and biology of Acrididae and in 1928 he had published *Locusts and Grasshoppers*, a lucid review of the whole field. His interest in ecology led to the publication in 1931 of *Insects and Climate*, which stimulated interest in a neglected field.

In the 1940s and 50s Uvarov travelled widely, visiting at least twenty-three countries at the invitation of governments and international organizations and, because of his encyclopaedic knowledge of the subject, he was able to make far-reaching suggestions for locust research and control. The international organizations for controlling the African migratory and red locusts were established and, since then, no plagues of these species have appeared —a striking proof of the validity of Uvarov's theories and their practical significance. Studies of the desert locust, however, showed that its outbreak areas were not restricted; it had an invasion area covering more than fifty countries. Uvarov saw that special coordination was necessary and interested the Food and Agriculture Organization of the United Nations which, in 1953, undertook the international coordination of control campaigns against the desert locust. Uvarov was appointed consultant to the FAO Technical Advisory Committee and was to play a major part, particularly as chairman of the FAO Panel of Experts on the Long-Term Policy for Desert Locust Control in 1956. He also helped to initiate the UNDP/FAO Desert Locust Project, the largest single project ever supported by the UNDP.

In 1959 he retired as director of the ALRC but remained a consultant, and began a new book on grasshoppers and locusts; he was as active as ever and, in 1959, toured Australia at the invitation of the CSIRO to give advice about the Australian plague locust, leading eventually to a large collaborative research project between CSIRO and the ALRC which is still in progress. He had long been a member of the UNESCO Arid Zone Committee and now began to explore a new field, expressed in a series of important papers on arid zone entomology and the entomological problems of agriculture in developing countries. Vol. 1 of *Grasshoppers and Locusts* was published in 1966 and he was working on Vol. 2 until shortly before his death.

Uvarov received many medals, awards and public honours; he was elected to the Royal Society in 1950 and knighted in 1961. He was a man of great strength and integrity of character, coupled with simplicity and humility. Sometimes he had a sharp tongue but it was tempered with a dry sense of humour; above all, he was always ready to help his fellow scientists in any way he could. He would wish nothing better for his memorial than that the work he began and fostered should continue and expand.

Correspondence

Cost of a Facelift

SIR,—The issue of *Nature* of April 25 contains an article about the delay in the resurfacing of the Arecibo reflector which is misleading (*Nature*, **226**, 308; 1970).

The costs of the resurfacing project are neither very uncertain nor have they suddenly risen greatly. The estimated cost has increased in a year from $3·8 million to $4·2 million, giving a percentage increase less than most estimates of the annual inflation in construction costs of this type. The firmness of the $4·2 million figure is supported by a formal fixed price bid at this figure from a competent company.

No significant engineering problems have been uncovered. There is no serious criticism of the proposed type of surface panel and no indication that a heavier surface would be necessary. The engineering of new surface on a foundation whose accuracy is already known is quite straightforward, and the major challenge is actually in determining the least expensive mode of fabrication of the large number of panels.

There is thus no reason for thinking that a much higher price estimate at some future time will arise and that this will require a re-evaluation of radio astronomy priorities. The resurfacing of the Arecibo reflector remains scientifically the most exciting and cost-effective foreseeable improvement to the world's astronomy equipment, and is one of the most promising projects of science in general.

Sincerely yours,

FRANK D. DRAKE

Cornell University,
Arecibo Project Office,
Space Sciences Building,
Ithaca, NY 14850, USA.

It is good to have Professor Drake's assurance, but it remains a fact that the National Science Foundation has undertaken to the Authorization Committee in the Senate that it will not expect to be awarded the money asked for on behalf of Arecibo until a further engineering study has been carried out. Everybody will hope that Professor Drake is right and he after all should know—he has done the work.—Editor, *Nature*.

Abortion Act in Action

SIR,—Your note on the Abortion Act (*Nature*, **225**, 580; 1970) quotes an estimate[1] of 18,000 private legal abortions in 1967, in addition to 10,000 under the National Health Service. Reliable figures for private terminations before the Abortion Act are certainly hard to come by, but there is unequivocal proof that this estimate must be exaggerated.

In 1967, 9,700 (not 10,000) NHS abortions were officially reported for England and Wales[2], so that another 18,000 privately procured would make a total of 27,700 pregnancies legally terminated in that year. But in the first 9·5 weeks under the new Act (April 27–July 2, 1968) 2,752 NHS and 1,660 private legal abortions were notified, corresponding to an annual rate of 24,036. Since it is not to be believed that the coming into operation of a new and more permissive law should have resulted in a significant reduction of the legal abortion rate, and since the NHS total is well established, it is the estimate for private abortions in 1967 which must be too high. Q.E.D.

If the 1967 rate had been the same as for the 9 weeks after April 27, 1968, there would have been no more than 14,356 private legal abortions in that year. But this also seems too high, and it is unlikely that the true figure could have exceeded 10,000, to give a total of 19,700 legal abortions in 1967. This would imply that the 1967 rate had been 18 per cent below what it was immediately after the new Act came into force, which is not excessive when compared with the 32 per cent increase in the next quarter (7,938 in the 13 weeks July 3–October 1), and a further 25 per cent (9,907 legal abortions) for the quarter ended December 31, 1968[3].

Your note also says that while there had been between 45 and 50 deaths from abortion each year for the past 20 or so years, this had dropped to 22 during 1969. But that is not so, since the figure of 22 was only for the 10 months ended October 31, 1969[4]. Mr Richard Crossman (Minister of State for the Social Services) has recently given[5] the provisional total for the full year 1969 as 29 deaths due directly to abortion, including 4 resulting from legally induced abortion (ICD 640 and 641), but not including a further 13 deaths after legal terminations of pregnancy under the Act which were not classified as directly due to abortion.

It may well be true that abortion deaths have averaged 45–50 over the past 20 years, but the rate is known to have been falling during this period, and for 1967, which was the last full year under the old law, the Registrar General[3] recorded 34 abortion deaths:

Abortion induced for medical indications (640)	1
Abortion induced for other legal indications (641)	0
Abortion induced for other [illegal] reasons (642)	17
Spontaneous abortion (643)	4
Abortion not specified as induced or spontaneous (644)	11
Other abortion (645)	1
Total abortion deaths	34

Even assuming that none of the 13 deaths following upon but not classified as directly due to legal abortions in 1969 would formerly have been counted as abortion deaths, and it is not entirely clear whether this would have been so, the improvement has been only from 34 deaths in 1967 to 29 in 1969. Perhaps 1967 may have been a better year than average, but your note in concluding that "The Act has been remarkably successful in reducing the death rate from abortion. The latest figures from the Ministry of Health show that in England and Wales the number of deaths from abortion, which has been between 45 and 50 per year for the past twenty or so years, dropped to 22 during 1969", does perhaps rather overstate the case.

Yours etc.,

C. B. GOODHART

Gonville and Caius College,
Cambridge.

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[1] Diggory, P., Peel, J., and Potts, M., *Lancet*, i, 287 (1970).

[2] Crossman, R., *Hansard (Commons)*, written answers, col. 15 (Nov. 10, 1969).

[3] Registrar General, *Quarterly Returns for England and Wales* (1968–69).

[4] Crossman, R., *Hansard (Commons)*, written answers, col. 197 (Feb. 5, 1970).

[5] Crossman, R., *Hansard (Commons)*, written answers, col. 272 (Mar. 23, 1970).

Announcements

University News

Dr R. W. Hockney, IBM Research Center, New York, has been appointed professor of computer science in the **University of Reading.**

In the **University of Sheffield, Dr D. A. Walker,** Imperial College, has been appointed professor of biology in the Department of Botany, and **Mr J. A. Thornton** has been appointed professor and head of the Department of Anaesthetics.

Mr Andrew Colin, University of Lancaster, has been appointed professor and head of the Department of Computer Science in the **University of Strathclyde,** and **Mr James G. Fraser** has been made director of computer services.

Dr J. E. Smith has been appointed to the new chair in microbiology in the Department of Biological Sciences, **University of Surrey.**

Appointments

Mr Philip Homan has been appointed to the post of director of the **Ministry of Technology's Office for Scotland** in Glasgow.

Mr N. S. Liverman, director of Normans Nurseries Ltd, has been appointed chairman of the governing body of the **Glasshouse Crops Research Institute,** in succession to the late Mr L. C. Madsen.

Miscellaneous

Lieutenant-Colonel F. J. Griffin has been awarded the **International Medal** of the Society of Chemical Industry. **Dr Charles Stark Draper,** Massachusetts Institute of Technology, has been awarded the **Founders Medal** of the US National Academy of Engineering.

Dr Donald J. LeRoy, National Research Council of Canada, has been awarded the **Chemical Institute of Canada Medal** for 1970.

The international journal *Organic Mass Spectrometry* has issued a **cumulative index of 2,200 chemical compounds** with special reference to those for which bar graphs or tabulator data have been published. The index is available free from Heyden and Son Ltd, Spectrum House, Alderton Crescent, London NW4.

Professor E. V. Shpol'skii has written to say that the English edition of his book *Atomic Physics* reviewed in *Nature* (**224**, 1135; 1970) was an incomplete translation of his Russian work. In the English edition it was not mentioned that the translation is of volume 1 only of the two-volume Russian edition. There is a German translation of the complete work available: *Atomphysik*, Teil 1 and Teil 2 (VEB Deutscher Verlag der Wissenschaften: Berlin, 1970).

The following elections have been announced by the **US National Academy of Sciences**: *Foreign Secretary,* Professor Harrison Brown, California Institute of Technology; *Members of the Council,* Dr Kingsley Davis, University of California, Professor James V. Neel, University of Michigan, Professor James A. Shannon, Rockefeller University, Professor Robert L. Sinsheimer, California Institute of Technology, Professor Clement L. Markert, Yale University; *Members,* Professor R. M. Adams, Dr B. J. Alder, Professor J. D. Baldeschwieler, Professor J. A. Berson, Professor P. D. Boyer, Dr L. M. Branscomb, Professor B. F. Burke, Professor S. A. Cain, Professor R. B. Corey, Professor J. W. Cronin, Professor W. G. Dauben, Dr E. E. David, Professor R. Emerson, Professor S. H. Emerson, Professor A. E. J. Engel, Professor R. A. Good, Professor R. M. Goody, Professor H. Grad, Professor J. D. Hardy, Professor L. A. Heppel, Professor H. Hotelling, Professor A. D. Kaiser, Professor N. O. Kaplan, Professor I. M. Klotz, Professor R. C. Lancefield, W. B. Langbein, Professor P. D. Lax, Professor D. S. Lehrman, Dr A. S. Leopold, Professor C. Levinthal, Professor M. S. Livingston, Professor W. J. Luyten, Professor R. A. Marcus, Professor C. V. Moore, Professor E. P. Odum, Dr R. Patrick, Professor G. L. Pearson, Professor A. Rich, Professor H. L. Roman, Professor P. A. Samuelson, Professor A. L. Schawlow, Professor M. M. Schiffer, Professor S. Smale, Dr G. D. Snell, Professor J. L. Strominger, Professor M. Tinkham, Professor B. J. Underwood, Professor B. Wallace, Professor A. White, Professor A. S. Wightman.

International Meetings

May 29, **The Use of CS and Other Harassing Gases in War: the Geneva Protocol Then and Now,** London (Professor P. J. Lindop, Department of Radiobiology, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1).

September 14–16, **Plastics Pipes,** Southampton (Symposium on Plastics Pipes, c/o Dr Howard Allen, Department of Civil Engineering, The University, Southampton SO9 5NH).

September 23–25, **Sporopollenin,** London (Symposium Organizing Secretaries, Department of Geology, Royal School of Mines, Imperial College, London SW7).

October 15–16, **Telecommunication,** Madison (TDAL and ETN/SCA Conference, Mrs Anne G. Niles, Department of Nursing, University Extension, 606 State Street, Madison, Wisconsin 53706, USA).

October 26–28, **Electronic and Aerospace Systems,** Washington (Technical Conference Services, IEEE Headquarters, 345 East 47th Street, New York, NY 10017, USA).

October 29–30, **Chemisorption and Catalysis,** London (Conference Secretary, Institute of Petroleum, 61 New Cavendish Street, London W1).

November 2–4, **Ceramic Machining and Finishing,** Gaithersburg, Maryland (NBS Office of Technical Information and Publications, Room A500/101, Washington, DC 20234, USA).

November 4–6, **Nuclear Science,** New York (Institute of Electrical and Electronics Engineers, 345 East 47th Street, New York, NY 10017, USA).

November 6–10, **First National Biological Congress,** Detroit (Dr M. Ben, American Institute of Biological Sciences, 3900 Wisconsin Avenue NW, Washington DC 20016, USA).

November 9–16, **Vision Week** at Ohio State, Columbus (Dr Glenn A. Fry, College of Optometry, Ohio State University, 338 West Tenth Avenue, Columbus, Ohio 43210, USA).

November 15–19, **Joint Meeting of the American Nuclear Society and the Atomic Industrial Forum and Atomic Fair,** Washington (Mr O. J. Du Temple, American Nuclear Society, 244 East Ogden Avenue, Hinsdale, Illinois 60521, USA).

November 17–20, **Magnetism and Magnetic Materials,** Miami Beach (G. P. Rodrique, Local Committee, Georgia Institute of Technology, School of Electrical Engineering, Atlanta, Georgia 30332, USA).

November 30–December 4, **Engineering Materials and Design,** London (c/o Industrial and Trade Fairs, Ltd, Commonwealth House, New Oxford Street, London WC1).

December 1–8, **Results of Research on Representative and Experimental Basins,** Wellington (Mr G. W. Markham, Royal Society of New Zealand, PO Box 196, Wellington, New Zealand).

December 6–11, **International Clean Air Congress,** Washington (Mr Arnold Arch, Executive Secretary, Air Pollution Control Association, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA).

December 14–18, **Relativistic Astrophysics,** Austin (L. C. Shepley, Physics Department, University of Texas, Austin, Texas 78712, USA).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Vacant: Furnished house in Chevy Chase, Maryland, close to NIH and most Washington and suburban Maryland hospitals and universities (American, George Washington, Howard, Catholic, and Georgetown). Three bedrooms, near elementary school, bus, shops, public park. One year lease, available June 15, 1970, $300 the month. Please contact Dr L. Lewin, 8218 Larry Place, Chevy Chase, Maryland 20015, USA (telephone 301–588–0982).

Wanted: Three-bedroomed, furnished house in Reading, Berkshire, for American professor, wife, 4 children. Prefer central heating. Will consider one year lease mid-August 1970 to mid-August 1971, up to £90 per month. Please contact L. Sequeira, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53705, USA.

Wanted: Furnished 3 or preferably 4-bedroom house with central heating, in Reading, Berkshire, for year beginning August 20, 1970. Please contact Dr Locke White, jun., Department of Physics, Davidson College, Davidson, NC 28036, USA.

British Diary

Monday, May 18

Avalanche Diodes—Normal and Subnormal (11 a.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

Studies on the Mechanism of Malarial Immunity (5 p.m.) Professor S. Cohen, University of London, at the Wright-Fleming Institute, St. Mary's Hospital Medical School, Norfolk Place, London W2.

The Effects of Metrication on Power Cable Specifications (5.30 p.m. discussion meeting) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Global Challenge (5.30 p.m.) Mr Thane Rhiney (Rome), University of London, in the Botany Theatre, University College London, Gower Street, London WC1. (First of three Special University Lectures in Conservation.)

Tuesday, May 19

Aeronautical Acoustics as a Problem in Applied Mathematics (5.30 p.m.) Professor J. E. Ffowcs Williams, University of London, at Imperial College of Science and Technology, London SW7. (Inaugural Lecture.)

British Postal Engineering Conference (three days) Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group, at 1 Birdcage Walk, London SW1.

Charles Darwin and his Circle (5 p.m.) Professor Sir Hedley Atkins, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

Excavations in Baba Jan, Luristan 1966–1969 (5.45 p.m.) Dr Clare Goff, University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Fluorescence with reference to Medical and Pharmaceutical Applications (4.30 p.m.) Mr J. W. Bridges, Society for Analytical Chemistry, jointly with Loughborough University of Technology Chemical Society, at the University of Technology.

Repetitive Activity in Nerve and Muscle (5.30 p.m.) Professor R. Stampfli (Saar), University of London, in the Physiology Theatre, University College London, Gower Street, London WC1.

Signal Processing Methods for Radio Telephony (three-day conference) Institution of Electrical Engineers, at Savoy Place, London WC2.

Wednesday, May 20

Against Nature : The Use and Misuse of Birth Control (5.15 p.m.) Dr D. M. Potts, Institute of Biology with the support of the Eugenics Society, in the Lecture Hall, Natural History Museum, Cromwell Road, London, SW7. (Tenth Darwin Lecture.)

Air Traffic and Navigation, Institute of Navigation, and the Royal Aeronautical Society, at 4 Hamilton Place, London W1.

Meteorology and Conservation (5 p.m. discussion meeting) Royal Meteorological Society, at 49 Cromwell Road, London SW7.

Numerical Computation of Aeronautical Structures by Finite Element Methods I (6 p.m.) Professor B. Fraeijs de Veubeke (Liège), University of London, at Imperial College of Science and Technology, London SW7. (First of four Special University Lectures in Aeronautical Engineering. Further lectures on May 21, 26 and 28.)

Research and Conservation (an Identification of Major Problems to be Solved) (5.30 p.m.) Mr Thane Rhiney, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Sonic Environmental Testing of Components and Equipments (6 p.m.) Mr D. Chadwick, Institution of Electronic and Radio Engineers, Instrumentation and Control Group, at 9 Bedford Square, London WC1.

The Psychiatry of Vagrancy (5.30 p.m.) Dr A. D. Isaacs, University of London, at the Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5.

Thursday, May 21

Arbovirus Epidemiology Associated with Established and Developing Rice Culture (7.30 p.m. symposium) Royal Society of Tropical Medicine and Hygiene, at 26 Portland Place, London W1.

Celestial Physics Laboratories (5.30 p.m.) Professor Sir Martin Ryle, FRS, University of London, at Senate House, London WC1. (John Coffin Memorial Lecture in Science.)

Profiting from Experience (a Demonstration of the Case Method as an Educational Tool) (5.30 p.m.) Mr Thane Rhiney, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Subcellular and Macromolecular Aspects of Synaptic Transmission (two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

The Human Factors in Management (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Friday, May 22

Research in Progress (10 a.m.) Department of Surgery, Royal Postgraduate Medical School, DuCane Road, London W12.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). **Zoology. Vol. 19 No. 1:** Notes on the Family Amphiuridae (Ophiuroidea). **By Ailsa M.** Clark. Pp. 1–81. (London: British Museum (**Natural History**), 1970.) 40*s*. [28

Building Research Station. Current Paper 50/69: **Loadings on Buildings—a Review Paper.** By G. R. Mitchell. Pp. 9. (Garston, Watford: **Building Research** Station, 1969.) *Gratis*. [33

Science Research Council: University Science and Technology Board. Differential Equations: The Interim Report of the Differential Equations Panel of the Mathematics Committee. Pp. 32. (London: Science Research Council, 1970.) *Gratis.* [33

British Antarctic Survey. Scientific Reports, No. 63: Some Aspects of the Anatomy of the Ross Seal, *Ommatophoca rossi* (Pinnipedia: Phocidae). By Judith E. King. Pp. 54+10 plates. (London: British Antarctic Survey, 1969.) 42*s* net. [33

University of Oxford. Report of the Committee on Extra-mural Studies. (Supplement No. 3 to the *University Gazette*, Vol. C, March, 1970.) Pp. 81. (Oxford: The University, 1970.) 7*s* 6*d*. [43

Industrial Discrete Devices. (Aylesbury: SGS (United Kingdom), Ltd., 1970.) 21*s*. [43

Higher Education Research in Australia and New Zealand: a Discussion of the Research Literature and other Studies. By Gordon W. Miller. Pp. 57. (London: Society for Research into Higher Education, Ltd., 1970.) 14*s* 6*d* (members 10*s*). [53

Memoirs of the Geological Survey of Great Britain. Palaeontology, Vol. IV, Part 6: Fossil Plants of the Carboniferous Rocks of Great Britain (Second Section). By Robert Crookall. Pp. xxix–xxxvi+793–840+plates CLI–CLIX. (London: HMSO, 1970.) 50*s* net. [53

Boundaries of Chemistry. By Professor D. H. Whiffen. (Inaugural Lecture delivered before the University of Newcastle upon Tyne on Monday, 3 March, 1969.) Pp. 14. (Newcastle upon Tyne: The University, 1969.) 5*s*. [63

Department of Education and Science. A Teaching Council for England and Wales—Report of the Working Party appointed by The Secretary of State for Education and Science. Pp. vi+41. (London: HMSO, 1970.) 4*s* 6*d* (22·5*p*) net. [63

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 257, No. 817 (5 March, 1970): The Middle Pleistocene Deposits at Marks Tey, Essex. By C. Turner. Pp. 373–440. (London: The Royal Society, 1970.) 37*s*; $4.80. [63

University of Birmingham—Research Committee. Research and Publications, 1968/1969, No. 40. Pp. 196. (Birmingham: The University, 1970.) [93

National Institute of Industrial Psychology. Annual Report and Statement of Accounts for the year ended 30 September 1969. Pp. 27. (London: NIIP, 1970.) [93

Royal Observatory Bulletins. No. 156: Motion of Ao Stars Perpendicular to the Galactic Plane. 3: Radial Velocities Observed at Kottamia. By Sir Richard Woolley, A. S. Asaad, M. P. Candy and Margaret J. Penston. Pp. 37–50. (London: Science Research Council, 1969.) 5*s* net. [93

Northern Ireland: Ministry of Agriculture. Leaflet No. 2: Farm Capital and Credit. Pp. 12. (Belfast: Ministry of Agriculture, 1970.) [103

1970 Holiday Addresses in England, Wales and the Channel Islands for Chest, Heart and 'Stroke' Patients. Pp. 40. (London: The Chest and Heart Association, 1970.) 4*s*. [103

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 266, No. 1172 (5 March, 1970): Periodic Forests of Stunted Trees. By J. C. P. Miller. Periodic Forests whose Largest Clearings are of Size 3. By H. G. Ap Simon. Pp. 63–121. 42*s*; $5.45. Vol. 266, No. 1173 (5 March, 1970): Uniqueness in the Inversion of Inaccurate Gross Earth Data. By G. Backus and F. Gilbert. Pp. 123–192. 37*s*; $4.80. (London: The Royal Society, 1970.) [103

The Carnegie Trust for the Universities of Scotland. Sixty-eighth Annual Report for the year 1968–69. Pp. 46. (Edinburgh: Carnegie Trust for the Universities of Scotland, 1970.) [123

Teacher Training for Science and Mathematics Graduates. (Report on a Conference held at the Royal Society at 17 October 1969.) Pp. 39. (London: The Royal Society, 1970.) [123

Ministry of Transport: Road Research Laboratory. RRL Report LR 302: 50-Point Traffic Census—Results for 1968. By J. B. Dunn. Pp. 7+16 tables. (Crowthorne, Berkshire: Road Research Laboratory, 1970.) *Gratis.* [123

Northern Ireland: Ministry of Agriculture. Leaflet No. 117: Potato Root Eelworm. Pp. 6. (Belfast: Ministry of Agriculture, 1970.) [133

Other Countries

Fisheries Research Board of Canada. Bulletin 170: Some Nearctic Podonominae, Diamesinae, and Orthocladiinae (Diptera: Chironomidae). By Ole A. Saether. Pp. ix+154. (Ottawa: Queen's Printer, 1969.) $3. [162

Report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended 31 March 1969. (H.21). Pp. 38. (Wellington: Government Printer, 1969.) 25 c. [162

Institut de France. Académie des Sciences—Annuaire pour 1970. Pp. 244. (Paris: Gauthier-Villars et Cie, 1970.) [162

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Smithsonian Contributions to Zoology. No. 17: The Taxonomic Status of the Controversial Genera and Species of Parrotfishes with a Descriptive List (Family Scaridae). By Leonard P. Schultz. Pp. v+49+8 plates. $1.25. No. 29: Deep-Sea Cerviniidae (Copepoda: Harpacticoida) from the Western Indian Ocean, collected with *R/V Anton Bruun* in 1964. By F. D. Por. Pp. 60. $0.65. (Washington, DC: Smithsonian Institution Press, 1969. For sale by US Government Printing Office.) [242

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APPOINTMENTS VACANT

NEW ZEALAND DEPARTMENT OF AGRICULTURE

The Animal Health Division is seeking recruits to its Veterinary Advisory Officer Service. Positions are available in various localities in New Zealand with headquarters in the larger provincial towns.

Positions are available specialising in infertility brucellosis; infectious diseases (2); metabolic disease; mastitis; and as Veterinary Advisory Officers (General). Appointments may be to the permanent staff or on a four-year contract basis.

Duties: Duties are broadly similar to those of Veterinary Investigation Officers in the United Kingdom. They include liaison with, and special services to practising veterinarians ; field investigation and surveys of disease incidence; development of disease control and eradication programmes; development of educational programmes for farmers on disease prevention ; field examination of new therapeutic products such as vaccines.

Qualifications: Minimum qualification M.R.C.V.S. or Degree in Veterinary Science. Preference will be given to those with post-graduate qualifications and previous field investigation experience and experience in epidemiology of exotic diseases. However, younger veterinarians with appropriate field experience who seek a career in the field of preventive veterinary medicine will be considered for appointment as Assistant Veterinary Advisory Officers. Developing Officers are given the opportunity for appropriate postgraduate training and training overseas in the recognition and control of exotic animal diseases.

Salary: Salaries for Veterinary Advisory Officers are in the range $NZ4,013 per annum to $NZ7,463 per annum. There are opportunities for advancement in administrative positions beyond this. For Assistant Veterinary Advisory Officers the salaries range from $NZ4,013 to $NZ5,248 per annum, advancing in due course through the Veterinary Advisory Officer scale. Starting salary in each case will depend on experience since graduation and relevant qualifications.

Expenses: Fares for appointee, wife and fully dependent children, including return fares for four-year contract appointees.

Interview, medical and passport expenses for appointee are payable by the Department.

A reasonable allowance is given for bringing light luggage to New Zealand, and assistance is given for furniture storage in United Kingdom (up to $NZ400) for contract employees.

Housing assistance is available to contract appointees.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, with whom applications will close on June 17, 1970.

Please quote reference B 13/2/170/1809 when inquiring. (1921)

NYEGAARD & CO. A/S
Pharmaceuticals and Technical Products

A vacancy exists for a

PHARMACOLOGIST

in our research department, where a small but enthusiastic staff of chemists, biochemists, pharmacologists etc. are tackling challenging problems in the pharmaceutical field. Our principal research interests at present are in the X-ray contrast and biochemical reagent fields.

Applicants should have a degree in pharmacology or related subject and some research experience: a higher degree would be no disadvantage.

Ability to speak Norwegian is not anticipated.

We can offer a good salary, an encouraging working atmosphere, 4 weeks holiday, national health and pension schemes and Norway's unequalled opportunities for outdoor life.

Applications as soon as possible to

Personell Department
Nyegaard & Co. A/S
Postboks 4220
Oslo, Norway.

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(1955)

CHEMIST/BIOCHEMIST

The Ministry of Defence requires a Chemist/Biochemist (graded Senior Scientific Officer/Scientific Officer) to join a group at the Microbiological Research Establishment, Porton, Wiltshire, whose principal function is to provide microbiol products for a wide range of research applications. The successful candidate will be expected to give leadership in the areas of extraction chemistry and analytical chemistry, especially in relation to enzymes. He will be encouraged to extend his interests to cover the full range of disciplines involved in the development of microbiol products. Publication of results is also encouraged.

Qualifications and Experience: Candidates should have a 1st or 2nd Class Honours degree, or equivalent in either biochemistry, microbiology or an appropriate related subject and for SSO at least three years' relevant post-graduate research experience. Knowledge and experience in protein chemistry, but especially in methods for the purification and assay of enzymes would be useful.

Superannuation under FSSU. Prospects of promotion and permanent pensionable employment.

Age: SO under 29; SSO normally 26–31.

Salary: SO £1,162–£1,982; SSO £2,193–£2,703.

APPLICATION FORMS from Mr. A. L. LYTTON, Ministry of Defence CE2(E)(AD) Room 302, Northumberland House, Northumberland Avenue, London WC2.

(1960)

ANALYTICAL RESEARCH

The Research Division of Pfizer Limited was created in 1957 and since that time has expanded steadily and now employs some 450 people, 200 of whom are scientists in various disciplines: chemistry, biochemistry, pathology, pharmacology, bacteriology, virology, parasitology, veterinary science and medicine. Its direct research expenditure is over £1.7 million in 1970.

We have a vacancy for a chemist in our Chemical Research Department whose main responsibility will be the design and development of appropriate analytical methods in the investigation of new research syntheses for novel compounds, and their subsequent preparation for new drug submissions to regulatory authorities.

The successful applicant will have a Ph.D. in analytical/physical organic chemistry, or be a graduate with not less than 3 years relevant experience. A good knowledge of organic chemistry and an ability to understand synthetic problems from the analytical viewpoint is essential. He should also have a reasonable knowledge of techniques such as GLC, TLC, UV, IR, NMR, Mass Spectrometry as applied to Organic Chemistry in general.

He will liaise closely with a large number of research staff in a team for attainment of research objectives of the Company. Attendance at scientific meetings and the publication of papers is encouraged.

The laboratories, modern and well-equipped, are situated on the Kent coast at Sandwich. There is a wide choice of residence in pleasant coastal or rural areas.

The starting salary will be competitive and the conditions of employment include pension and death benefit scheme, bonus scheme and the payment of removal expenses, if appropriate.

Applications, which will be treated in the strictest confidence should be addressed to:

D. W. Sells,
Personnel Manager,
Research Division,
Pfizer Limited,
Sandwich, Kent.

(1993)

CITRUS ENTOMOLOGIST

SWAZILAND

The Swaziland Citrus Board invites applications for the post of citrus entomologist, to continue research on the biology and control of the major pests of citrus in Swaziland. The citrus research unit is based at the central research station of the Department of Agriculture at Malkerns.

Applicants should have a B.Sc. in zoology or entomology with some postgraduate research experience in agricultural entomology.

Salary according to qualifications and experience in the range R3,200 to R4,500, or higher in exceptional cases. £1 sterling = R1.71. The initial contract is for 3 years, with gratuity of 25% on completion of contract, which may be renewed for further terms of 3 years. Fares paid for appointee and dependants. Modern house at sub-economic rental. Free ordinary medical attention is provided for appointee and family.

Applications giving details of education and experience, with the names of two referees, should be sent to:

The Secretary, Swaziland Citrus Board, P.O. Box 343, Mbabane, Swaziland, as soon as possible.

(1828)

NORTH EAST LONDON POLYTECHNIC

ROMFORD ROAD, STRATFORD, E.15

DEPARTMENT OF POSTGRADUATE MOLECULAR BIOLOGY

Applications are invited for appointments as LECTURER/SENIOR LECTURER within this newly established Department. There are five vacancies in the particular fields detailed below:

Molecular Biology
Biochemical Genetics
  Preferably concerned with nucleic acid and protein biosynthesis.
Cell Biology
  with special reference to tissue culture.
Protein Biochemistry
  fractionation and identification.
Cellular or Genetic Immunology

Although candidates with research interests in these fields are preferred, others will be considered. All appointees will be expected to participate in research within the Department and in organising and teaching in the Postgraduate courses. Good research facilities are available.

Salary Scale:
  Lecturer Grade I, £1,315 to £2,285 per annum.
  Lecturer Grade II, £2,032 to £2,622 per annum.
  Senior Lecturer, £2,622 to £2,957 per annum.
  (including London Allowance).

Details and Application form from R. P. Hornung, Chief Administrative Officer, North-East London Polytechnic, Romford Road, London, E.15. (1932)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF CHEMISTRY

Applications are invited for two POSTGRADUATE awards (S.R.C. rates plus fees) for research in the field of organophosphorus chemistry. Candidates would be expected to register for the degree of Ph.D. (University of London) and should have, or expect to have, a good B.Sc. degree or equivalent qualifications. The Department is fully equipped with modern instruments (including n.m.r. and e.s.r.) and attendance at postgraduate summer courses would be supported and encouraged.

Applications (including curriculum vitae and the names of two referees), should be sent, as soon as possible, to Dr. C. D. Hall (c/o Professor D. H. Hey, F.R.S.), Department of Chemistry, King's College London, Strand, London, WC2R 2LS.

(1934)

UNIVERSITY OF SOUTHAMPTON

INSTITUTE OF SOUND AND VIBRATION RESEARCH

LECTURESHIP IN STRUCTURAL DYNAMICS

Applications are invited for the post of Lecturer in the Institute of Sound and Vibration Research. Applicants should have experience and research interests in the field of structural dynamics. The person appointed will be expected to assist in the undergraduate and postgraduate teaching in the Institute and also to develop research work in one or more aspects of structural dynamics. Salary scale: £1,585 to £2,850 with a merit bar at £2,045.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (seven copies from United Kingdom applicants) should be sent by not later than May 30, 1970, quoting ref.: N. (1922)

UNITED NATIONS

seeks

Science Affairs Officer

Applications invited from persons with scientific background, for appointment to the Office for Science and Technology. Duties include preparation of reports on various aspects of the application of science and technology to problems of economic and social committees. The work of the office is closely associated with that of the United Nations specialized agencies and IAEA as well as with other inter-governmental and non-governmental organizations.

High university degrees desired in such fields as human biology, nutrition, computer technology and applications, environmental and oceanographic sciences, nuclear energy and engineering research, some background in the social sciences (preferably economics) and an interest in and familiarity with the conditions and problems of developing countries. Capacity to write clear English in relatively non-technical terms highly essential; additional knowledge of French highly desirable.

Starting Salary is in range of $13,980–$17,140 plus substantial cost-of-living adjustment. Benefits include six weeks paid vacation per year; medical and dental insurance and a pension plan.

Address replies to:

Secretariat Recruitment Service,

United Nations, New York, N.Y. 10017

(2031)

ELECTRON MICROSCOPE TECHNICIAN

A Senior Technician or Technician is required to assist in work on the physical biochemistry of muscle proteins. Electron microscopy forms a large part of the work, and some experience in this technique is essential. Salary on the scale (currently under review): Technician Minimum: £743, Maximum: £1,127 per annum. Senior Technician Minimum: £1,126, Maximum: £1,361 per annum.

Application to Dr. A. J. Rowe, School of Biological Sciences, University of Leicester, Leicester. (1964)

UNIVERSITY OF MANCHESTER

DEPARTMENT OF CHILD HEALTH

RESEARCH ASSISTANT required to join newly-formed group studying hormonal control of foetal growth. Zoological, physiological or biochemical background to II 1 or I class honours degree desirable. Study for a higher degree encouraged. Salary up to £1,355 per annum. Duties begin July 1, period of appointment to be negotiated.

Particulars and application forms (returnable by May 30) from the Registrar, The University, Manchester, M13 9PL. Quote ref. 102/70/Na. (1979)

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for (a) SENIOR LECTURESHIPS, (b) LECTURESHIPS IN DEPARTMENT OF PHYSICS. Appointments will be made at the Senior Lecturer level where possible, and preference will be given to applicants with research experience in the fields of ionospheric physics, geomagnetism, applied geophysics, or the physics of the lower atmosphere. Salary scales: (a) £N2,275 to £N2,575 per annum. (b) £N1,200 to £N2,175 per annum. (£N1 = £1 3s. 4d. sterling). Salary supplemented in range £450 to £725 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate cases by British Government. F.S.S.U. Family passages; various allowances; regular overseas leave.

Detailed applications (six copies), naming three referees, by June 26, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, W1P ODT, from whom particulars are available. (2021)

UNIVERSITY OF DUBLIN
Trinity College

School of Veterinary Medicine

CHAIR OF CLINICAL VETERINARY PRACTICES

Applications are invited for the above post, which will fall vacant in October, 1970. The Professor in Clinical Veterinary Practices is head of the department embracing Veterinary Medicine, Veterinary Surgery, Veterinary Obstetrics and Reproductive Diseases, Animal Husbandry and related disciplines. Further information may be obtained from the:

Secretary to the College,
Trinity College,
Dublin 2.

Closing date for the receipt of formal applications is June 13, 1970. (2015)

UNIVERSITY OF LONDON

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

COURTAULD INSTITUTE OF BIOCHEMISTRY

A graduate Biochemist is needed to undertake a study of the Biosynthesis of Plasma Lipoproteins of the role of the Liver in maintaining plasma lipoprotein patterns. The appointment, initially for three years, will provide opportunities for study for a higher degree. Salary, subject to review, within the scale £860 to £1,010 and superannuation under the F.S.S.U.

Applications to Dr. Mills, Courtauld Institute, The Middlesex Hospital Medical School, London, W1P 5PR. (1965)

UNIVERSITY OF STRATHCLYDE

Department of Economic History

LECTURESHIP IN HISTORY

Applications are invited from suitably qualified candidates for the above post. Candidates should have special competence in the field of late mediaeval and early modern European History.

Salary scale: £1,355 to £3,105 per annum, with F.S.S.U. Placing on scale according to qualifications and experience.

Application forms and further particulars (quoting 28/70) can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.I, with whom applications should be lodged by May 29, 1970.

(2016)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSICS

Applications are invited for the post of RESEARCH ASSISTANT to Professor N. H. March, tenable for two years in the first instance and supported by a Science Research Council Grant. Work will be on phonon theory in liquids and solids. Commencing salary in the range £1,240 to £1,815, dependent on qualifications and experience. Applicants should have a Ph.D. or equivalent research qualifications in Theoretical Physics.

Applications to the Registrar by June 8, 1970. Quote Ref.: R.39/G. (1991)

The Polytechnic of the South Bank

(Proposed)

FACULTY OF ENVIRONMENTAL SCIENCE & TECHNOLOGY

The Faculty is concerned with the scientific specification, design and engineering of the environment for man, his food and his industry. This involves the closest collaboration between scientists, engineers, architects, public health officials, industrial medical officers and managers. The Faculty will continue the teaching and research work of the National College for Heating, Ventilating, Refrigeration and Fan Engineering in this developing field—new degree and postgraduate courses are also being planned.

It is intended to fill the following new posts in September 1970:

PRINCIPAL or SENIOR LECTURERS in

Environmental Technology of Buildings (N.1)
(with special interest in the Total Energy Concept in the design of building services)
Environmental Physics (Heat Transfer) (N.2)
Environmental Physics (Acoustics) (N.3)
(Two Principal Lectureships are available to candidates with appropriate experience and qualifications)

SENIOR LECTURERS in

Applied Heat Transfer (Buildings) (N.4)
Heating and Ventilating Contracting (including Air Conditioning) (N.5)
Heating Plant (Buildings) (N.6)
Environmental Pollution (N.7)

LECTURER (Grade II) in

Refrigeration Plant and Applications (N.8)

Salary Scales (plus in each case £120 p.a. recently approved by the Burnham Education Committee)

Principal Lecturer—£2,767–£3,107
Senior Lecturer—£2,502–£2,837 Lecturer II—£1,912–£2,502

RESEARCH ASSISTANTS are also required for work on—

(a) the operational characteristics of air conditioning systems;
(b) the turbulent mixing of air;
(c) the interaction of ventilation and noise in air conditioned offices.

Further details and application forms, quoting reference, from The Clerk to the Governing Body (Room 407), Borough Polytechnic, Borough Road, London S.E.1. (Closing date 29 May, 1970.) (1985)

CANADA

GOVERNMENT OF SASKATCHEWAN

PRECAMBRIAN GEOLOGISTS (M.Sc. or Ph.D.) to lead five man summer field parties in geological mapping in the Precambrian Shield area of Saskatchewan and after laboratory studies to prepare reports for publication.

SEDIMENTARY GEOLOGIST (Honours Degree or Degree in Geological Engineering) to pick formation boundaries from mechanical logs, examine cores and samples and prepare maps, cross-sections and reports for publication.

GEOPHYSICIST (Graduate in Geophysics or equivalent) for checking incoming data, inspecting field operations, organizing studies for departmental use and publication and reviewing regulations. Qualifications must include professional experience in exploration work with emphasis on interpretation.

Satisfactory commencing salaries will be paid depending upon experience and training.

Please contact Mr. W. C. A. Knights, Immigration Department, Government of Saskatchewan, 28 Chester Street, London, S.W.1 (01-235-1871). (1980)

UNIVERSITY OF DUNDEE

Applications are invited for the post of RESEARCH FELLOW in the DEPARTMENT OF BIOLOGICAL SCIENCES from August 1, 1970, or as soon as possible thereafter. The successful candidate, who is expected to have a higher degree involving entomology, will work under the direction of Dr. F. L. Waterhouse, in stored products entomology with particular reference to insect behaviour. The appointment will be for one year in the first instance. Salary in the region of £1,470 per annum.

Applications (three copies), containing the names of two referees, should be sent by May 30, 1970, to The Secretary, The University, Dundee DD1 4HN. (1881)

THE BRITISH COUNCIL

BRAZIL

THE TECHNICAL INSTITUTE OF AERONAUTICS, SAO JOSE DOS CAMPOS, SAO PAULO requires TWO PROFESSORS / ASSOCIATE PROFESSORS specialising in (a) INTERNAL COMBUSTION AND GAS TURBINE ENGINEERING and (b) THERMAL POWER PLANTS by July, 1970, or as soon after as possible. Candidates should have a degree from a university in Britain and (for post (a)) experience in Engine Combustion problems or hydrocarbon combustion in general, and (for post (b)) experience of research in flow problems in engines or of engineering applications of compressible flow problems. For both posts a Ph.D. degree, and some knowledge of Portuguese would be an advantage.

Salary: £2,850 to £3,750 per annum according to qualifications and experience. Married oversea allowances within the following ranges: accompanied £591 to £320, unaccompanied £406 to £135. Children's and home education allowances. Income tax refunded if levied. Free furnished accommodation. Outfit grant. Employer's superannuation contribution paid. Medical and insurance schemes. Fares paid for Professor and family, including mid-tour passages for children in Britain. Assistance with freight on approved car. Two-year contract guaranteed by the British Council. 70/OU80-81.

THE TECHNICAL INSTITUTE OF AERONAUTICS, SAO JOSE DOS CAMPOS, SAO PAULO requires a VISITING PROFESSOR OF TELECOMMUNICATIONS by July, 1970, or as soon after as possible. Candidates, preferably men, should have a degree from a University in Britain, a postgraduate qualification, and professional and teaching experience. Some industrial experience would be an advantage.

Salary: £2,850 to £3,750 per annum according to qualifications and experience. Married overseas allowances within the following ranges: accompanied £591 to £320, unaccompanied £406 to £135. Children's and home education allowances. Income tax refunded if levied. Free furnished accommodation. Outfit grant. Employer's superannuation contribution paid. Medical and insurance schemes. Fares paid for Professor and family, including mid-tour passages for children in Britain. Assistance with freight on approved car. Two-year contract guaranteed by the British Council. 70/OU79.

Write, quoting relevant reference number, to Appointments Division, The British Council, 65 Davies Street, London W1Y 2AA, for further particulars and application form to be returned completed as soon as possible. (1914)

EDITORIAL ASSISTANT REQUIRED TO edit and prepare for printing manuscripts dealing with the science and technology of metals and alloys, to correct printers' proofs, and to deal with routine editorial matters. Knowledge of English usage and of scientific or technical terminology essential, metallurgical background desirable. Salary not less than £1,250 per annum.—Apply in writing to the Institute of Metals, 17 Belgrave Square, London, S.W.1. (2007)

NATURE

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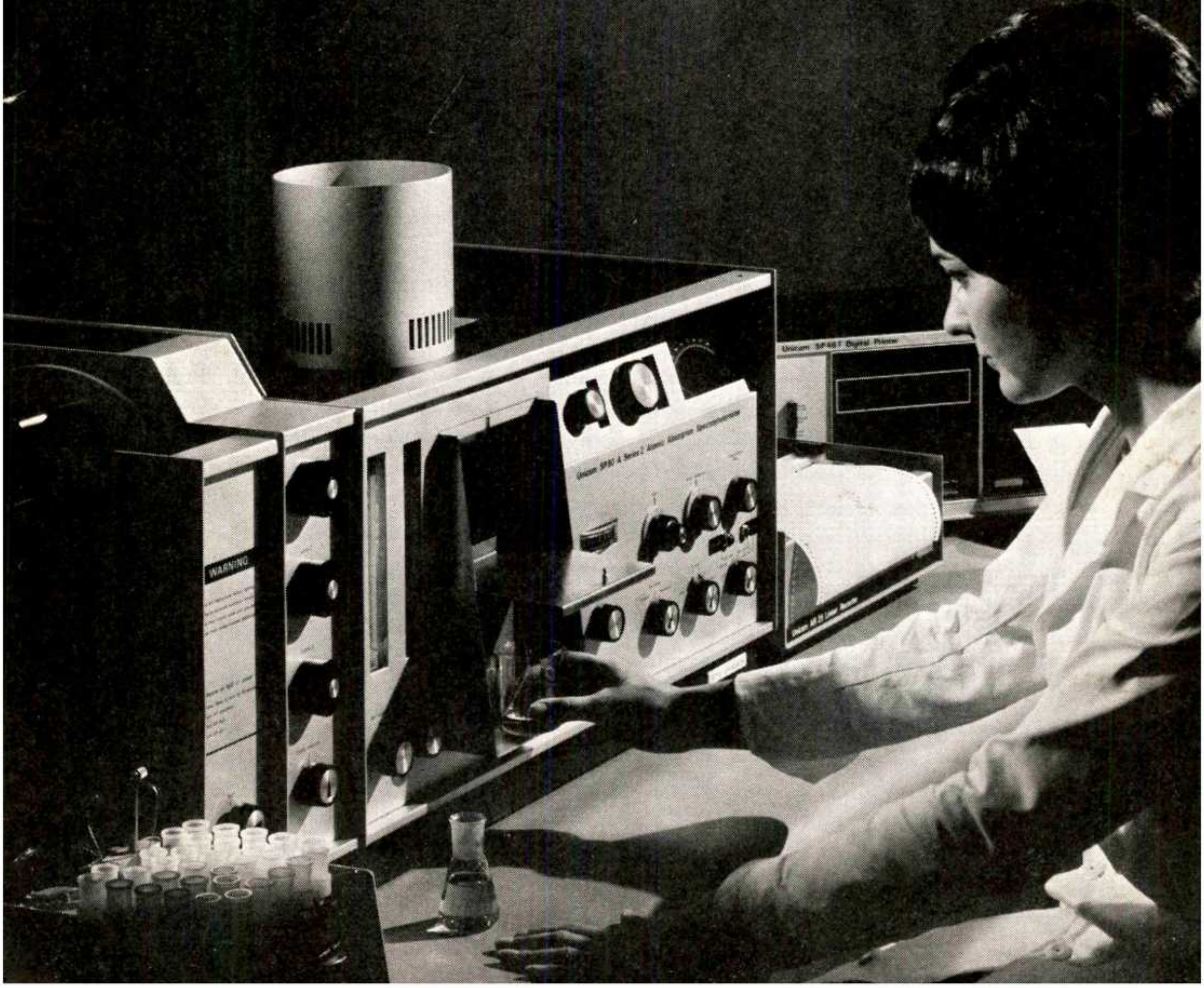

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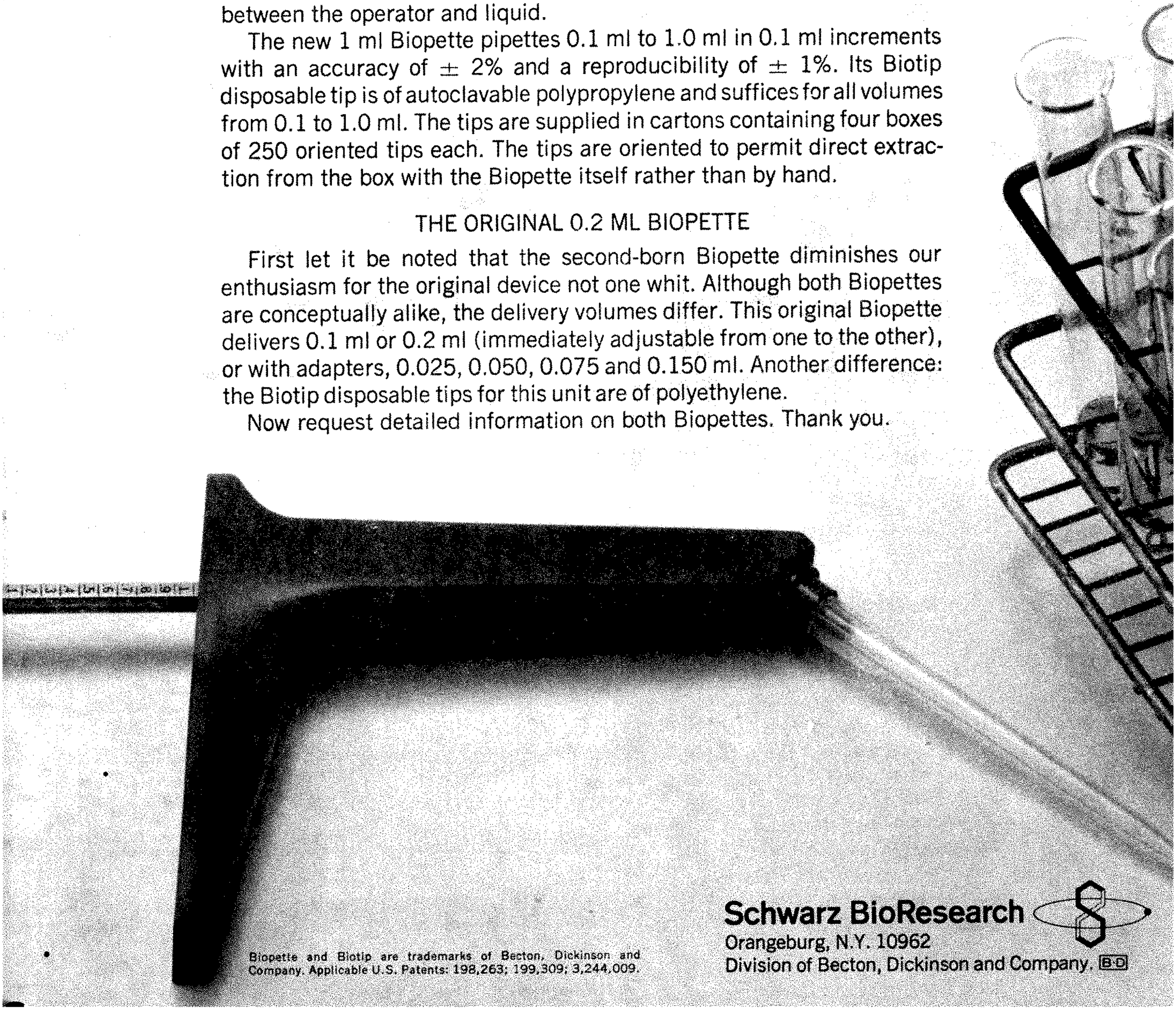

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NATURE Volume 226
MAY 23, 1970

Science is Neutral this Time

THE election which the British Prime Minister has arranged for June 18 will differ markedly from that of six years ago, when Mr Harold Wilson was first elected to office. Then the great cry was that what he called the "white-hot technological revolution" would make it possible for Britain to become a much more prosperous place. Since then, everybody—Mr Wilson included—has learned that rapid industrial change is a good deal more difficult than that. For much of the past six years, moreover, the anxiety which he has shared with a good many of those who elected him is whether the British economy would survive at all. The election has come out of the blue because the prospects, for the time being at least, are good. The Conservatives may complain, during the weeks ahead, that the economic indicators will take on a more sombre appearance in the autumn, when wage and salary increases will begin to take their toll and when the troubles of the United States economy may have an influence in Europe, but there is no doubt at all that Mr Wilson's strongest card in the election ahead will be his steadfast reputation for saying that everything would come right in the end. For the time being, at least, there is no sign that bankruptcy is around the corner. It is only natural that Mr Wilson and his colleagues should ask for another spell in office as a reward. This time, in all probability, very little will be heard of the technological revolution.

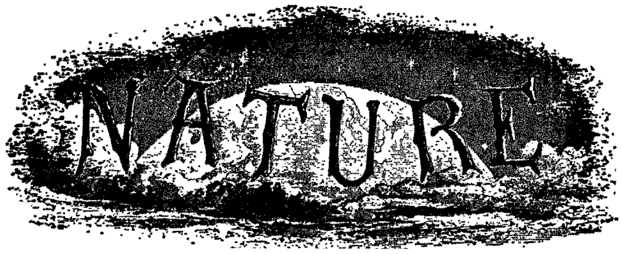
A complete moratorium on technical discussions in the weeks ahead will, however, be a misfortune, for there are several matters which need to be discussed. The Ministry of Technology's green paper on the reorganization of public science is one obvious issue which could usefully be clarified if not resolved during the election campaign. This might also be an occasion on which to press the British Government and its would-be successor for a clearer statement of policy towards the universities. Is it, for example, now commonly agreed that there will be no great expansion of graduate education in the years ahead ? How much weight must be given to the impression that both of the major British political parties find this item in their annual budget something of an embarrassment ? If the white-hot technological revolution has not happened, nobody will dispute that there has been a transformation of the Labour Government's expectations of science and technology.

Fairness therefore requires it to be understood that the government has done a sensible job in the management of basic research in the past six years. The apparatus of the research councils has worked smoothly and well. The Science Research Council in particular has been able to graft onto its traditional functions more adventurous activities. To be sure, among some academics there is now a measure of surprise that the Science Research Council's declared policy of concentrating support for particular fields of research on a few institutions, originally acclaimed, should now have turned out to be painfully effective, but this is only a measure of its success. In the relationship between higher education and research, the retiring government's most serious weakness has been the attempt to build up in parallel with the university system the whole apparatus of the polytechnics—it remains to be seen whether it will be possible for these two systems to coexist without an almost endless waste of resources and of young people's hopes and talents. No harm would come if the government were stimulated by the discomforts of a general election to think again about the wisdom of its arrangements for the development of polytechnics. At the same time, there is every reason why it should be reminded that there is still important work to be done in the further development of the research councils. There would be advantages in concentrating university relations in one of the councils, presumably the Science Research Council, freeing the others for more practical objectives. It would also make sense if some of the money at present provided by the University Grants Committee for university research were directed through the somewhat competitive channels of the research councils—in the long run that could be one of the most powerful incentives to innovation, uncomfortable though it might be for the universities.

Policy on industrial research is much more controversial, and the green paper on the organization of some of the government laboratories, intended as a focus for public discussion, is bound to become a focus for moderate dissension. The proposal is that the research and design establishments of the Atomic Energy Authority, together with some of the public laboratories such as the National Physical Laboratory, should be turned into a single organization living in part off public funds and in part off what they can raise by jobbing out their services either to government departments or to industry. The most obvious defect of the scheme is that the laboratories do not include those working on defence research, essential if the new British Research and Development Corporation is to function well in the fields in which there is most useful work to do—telecommunications, for example. By itself, this should be enough to make the project founder. But there are also problems about the extent to which the new corporation will actually be able to sell its services. The way in which it is likely to be composed, it is bound to seem to many people a pale shadow of the Atomic Energy Authority, itself in recent years a pale shadow of its former self.

In these circumstances, nobody should be surprised that the Conservative Party would take a more drastic line with those research establishments which have outlived their original functions, promising the men and women concerned monetary incentives to retire early, and would at the same time sublet much more of government research to industrial companies. There is a great deal in the arguments which have led to the formulation of this policy. The weakest part of the government's case is that nobody knows at present just what kind of work the British Research and Development Corporation would carry out. It is still too much a piece of imagery, and it would be a public service, since there has to be an election after all, if the occasion could be used to force the government to provide a more detailed prospectus of its intentions.

100 Years Ago



AN article of food has lately been introduced which has found its way into every grocer's and chemist's shop in the country, and for which there is in all parts of the world a vast demand. This substance is variously called the Extract of Meat, the Juice of Flesh, Liebig's Extract, and in Latin, *Extractum Carnis Liebigii.* The name of Baron Liebig, the great chemist, is more especially connected with this compound, as he has undoubtedly the merit of having first called attention to it as a valuable article of diet. In his "Familiar Letters on Chemistry," he devotes a letter to vegetable and animal food, and gives an account of their various chemical components. He shows with regard to all animal flesh, that besides fibrine, albumen, gelatine, and fat, it contains certain other constituents which may be separated from these by a simple process of infusion, straining, and evaporation. The substance thus obtained is the extract of flesh.

From Nature, **2**, 62, May 26, 1870.

OLD WORLD

FISHERIES

Compromise over Salmon

MOVES by Britain and other salmon breeding countries to ban the fishing of salmon in the North Atlantic have for the moment been defeated. At a meeting of the North-East Atlantic Fisheries Commission (NEAFCO) in London from May 6–11, the delegates decided instead to limit fishing in general to the period between May 5 and July 1 each year, and to ban it altogether only in two small areas to the north of Norway. The agreement does not cover the most important high seas salmon fishing grounds, which are off the coast of Greenland; next month, however, the meeting of the International Commission for North-West Atlantic Fisheries (ICNAF) is expected to reach substantially similar conclusions for the Greenland area, because the member countries of NEAFCO and ICNAF are largely the same.

Denmark has been the most vociferous opponent of a complete ban, urged chiefly by Britain and Canada because their freshwater and inshore salmon catches are, they claim, being threatened by Scandinavian drifters which pirate the salmon on the high seas. At the NEAFCO meeting last year, a proposal to stop high seas fishing of salmon for ten years failed to gain a big enough majority for acceptance. The opponents —Denmark, Sweden and West Germany—came out this year in favour of restrictions which, as well as imposing a forty-five week close season, prohibit the catching of fish smaller than 60 cm and limit the kinds of net that can be used. Britain and Iceland voted against the restrictions, but the general feeling at the meeting was that it was better to take advantage of the partial concessions by Denmark, Sweden and West Germany than to continue in support of radical measures whose adoption would again be made impossible by these three countries.

Unless any country objects within ninety days, the agreement becomes binding from the beginning of 1971, and will be reviewed after two years. As they stand, the restrictions are not as severe as they may seem. For one thing, there is already a natural close season for salmon in their high seas feeding grounds because they spend much of the year returning to their home rivers to breed. More important, there is no limitation on the number of boats that can exploit the salmon, so that catches could be kept up by involving more fishermen. This settlement, however, is probably all that can be achieved for the moment without damaging international relationships—between Britain and Denmark in particular—and does at least acknowledge the lack of direct evidence for a fall in coastal and freshwater catches. Should stronger evidence of diminishing stocks emerge, one course of action that has been suggested in several quarters is a boycott of Danish exports.

Unlike the Atlantic salmon, the North Sea herring has been quite clearly overfished in the last few years, and in several areas there are simply no herring left to be caught. The dangers inherent in the more capacious nets that have been used since 1965 were recognized by the NEAFCO meeting, which decided that herring should not be fished in the North Sea during May and from August 20 to September 30.

This is the first time that catches of herring have been limited internationally; the measures are provisional pending the negotiation of a quota system to halve the annual harvest. Herring are far more important economically than salmon, but the salmon problem has been causing most of the controversy because of the money that goes into breeding and conservation and the value of the fishing rights in salmon rivers. No single country can be blamed in isolation for the overfishing of herring, although the Scandinavian countries have large herring industries, and Russian and Polish ships have of late captured a growing proportion of the fish.

GOVERNMENT RESEARCH

What to Do about Harwell?

THE Confederation of British Industry has now come up with its comments on the plan by the Ministry of Technology for the reorganization of government research laboratories. Widely seen as an attempt to solve the problem of what to do with the laboratories of the Atomic Energy Authority now that the initiative is being shifted to the nuclear power companies, the government plan involves the setting up of what is being called the British Research and Development Corporation. This would be made up of the Harwell and Culham establishments of the AEA, the reactor group, five of the research laboratories of the Ministry of Technology occupied with civil research, and the National Research Development Corporation.

What the CBI dislikes about the plan is that it will not encourage the right kind of links between the laboratories and the industries which the ministry would like to see the laboratories provide. Obviously, the CBI believes that the closer research and development work is done to the point of application the better, with industrial laboratories as the ideal place. But, unlike other organizations which have been criticizing the plan since its publication as a green paper in January, the CBI has some alternative suggestions.

It identifies as the most urgent problem the need to deal humanely with the excess staff in the Atomic Energy Authority, left over from the time of intensive research into atomic energy. The CBI recommends several steps which might be taken, including the establishment of a working group to help with redeployment, which would take into account the future requirements of the Atomic Energy Authority as defined by the Atomic Energy Board which Mintech proposes. Government laboratories, research associations and educational establishments are seen as the likeliest places where the excess staff might find work, but the CBI stresses that the government has important financial responsibilities to those who go outside government service.

The CBI would like to see what is left of the research and development resources of the authority become a research association serving the nuclear power industry. The non-nuclear activities which the government has been encouraging in the authority would then have to be run separately by the Ministry of Technology.

Leaving aside the Atomic Energy Authority, the CBI has considered whether the five research establishments of the Ministry of Technology included in the green paper ought to be placed in a smaller version of the BRDC. But the conclusion is that the disadvantages of having in the same corporation five laboratories working in different fields outweigh the advantages, and the CBI prefers a modest reshuffling of the laboratories among government departments. The Ministry of Technology should retain responsibility for the National Engineering Laboratory, the National Physical Laboratory, the Warren Spring Laboratory (although here there is a need for links with the Ministry of Housing and Local Government), and the Forest Products Research Laboratory (but in this case the CBI admits there is a case for transfer to the Ministry of Public Building and Works). But the CBI joins with the Institution of Civil Engineers in recommending that the Hydraulics Research Laboratory become the responsibility of the Natural Environment Research Council, on account of the work on conservation undertaken by the laboratory.

The final component of BRDC, the National Research Development Corporation, whose job is to encourage the application of government and private research, ought to be seen to be independent of any large organization such as BRDC, and the CBI paper says there seems to be no reason why NRDC cannot continue as it is.

But running through all the discussions of the government's plan, including the latest important contribution from the CBI, is the fear that BRDC will be a cover-up for the running down of laboratories which do not pay their way. This is because the Ministry of Technology has set the target of BRDC being self-supporting by 1975, by earning what most critics believe is a hopelessly ambitious proportion of its annual cost of £70 million from private industry. The CBI's comment is that it would be wrong for the new corporation to be faced with a major redundancy in its staff as one of its first problems.

UNIVERSITY GRANTS COMMITTEE

Pays Piper but Calls No Tune

BRITISH universities have more academic freedom than their counterparts in any other country, and they are better off economically under the present system of allocating grants than they would be under any other system. The University Grants Committee can, of course, take most of the credit for this situation, because it places itself between the universities and their chief source of finance, allowing them to tread their own academic paths without having constantly to bow down to some outside authority.

This tribute which, when set against most utterances about academic freedom, seems notably uncontroversial, was made last week by Sir John Wolfenden, himself a past chairman of the UGC, in a lecture given to the Royal Society of Medicine. The conclusion seems a long way removed from the hysteria which has been stirred up by the recent allegations of industrial control at the University of Warwick.

Compared with universities in the United States, British universities can count themselves lucky. Sir John painted a vivid picture of presidents of private universities in the US travelling the country to drum up funds from business corporations and foundations, so that their research can be conducted free from any federal strings. But, said Sir John, "it is idle to pretend that funds which come from these sources are without strings", and he went on to give the advice to those attempting to establish an independent university that "it is an illusion to suppose that a private university is, by being private, academically independent. And those who contemplate establishing one in Britain should take to heart the experience and advice of their American colleagues before they embark on a will-o'-the-wisp enterprise here".

At the other extreme, American state universities are, according to Sir John, firmly in the pocket of the state government. The university president has to spend his time lobbying the state legislators to try to persuade them to do what he would wish to see done or, more often, to try to dissuade them from taking decisions which would undermine the academic freedom of his institution even further.

It is only the existence of the University Grants Committee that has prevented a similar situation occurring in Britain. The UGC acts as a buffer between the universities and the government so that, although the government subsidizes the universities to the tune of about £250 million a year, it keeps its fingers out of the internal affairs of each institution. "The government as such and the universities as a body never meet to argue about money, still less about the universities' internal affairs; between the two stands the UGC, ready to have its bottom kicked by both." But the situation is changing. The UGC can no longer be merely a go-between but must take an overall view of the situation, and plan its strategies accordingly. Centres of excellence should be built up in a few places, and duplication of effort avoided wherever possible. And there is nothing wrong with the UGC or the comptroller and auditor general encouraging the universities to be more economical.

What of the future? Sir John was remarkably reticent when it came to making predictions, but he did finish up on a cautionary note. "There is a danger," he said, "that a government might wish to take over the universities and make them the instrument of some policy or other which might be totally antagonistic to the academic freedom in which we all believe. That has happened elsewhere, and it would be naive to suppose that it could never happen here. More insidiously, because less obviously, Parliament, acting through a select committee, believing itself to be responding to public opinion, might exert pressure on a government to ensure that the universities did what they were told." But such a development, he promised, would only take place over the dead body of the UGC.

FOOD

Making Mincemeat of Starch

A CHEAP way of making protein from carbohydrates, under development by Ranks Hovis McDougall Ltd (RHM), has proved promising enough to attract backing worth £500,000 from the National Research Development Corporation. The key to the process is a fungus that can grow on carbohydrate residues, and the product, known as A3/5, is claimed to have the nutritive value of milk protein. A team at the RHM Lord Rank Research Centre in High Wycombe has already used A3/5 as the basis of a simulated meat, which is not yet entirely palatable but does at least lack the unpleasant aftertaste of many other protein concentrates, particularly those from soya.

The fungus is a strain of *Penicillium notatum*, but RHM will not reveal what the strain is until the process has been patented. According to Professor Arnold Spicer, director of research, RHM has a three-year lead over its nearest rival (a Japanese company) and the chief competitive advantage of A3/5 over proteins from other sources is that it will eventually sell at something like half the price. The National Research Development Corporation is equally enthusiastic and, although it has gone to some lengths in looking at other attempts to produce edible proteins, it is not planning to finance any more projects in the near future.

Almost any carbohydrate source can be used to make A3/5. One incentive for developing the process was that RHM wanted to find a use for its waste starch, although full-scale factories would presumably exploit local crops such as potatoes or cassava. A small plant which RHM is to open at High Wycombe next year will be starting from milk whey and molasses, together with nitrogen in the form of ammonia, and is intended to convert six tons a week of carbohydrate into three tons of A3/5. The output will be used for large-scale trials on farm animals; evaluation on humans is not planned until 1973.

The making of the protein is one thing; the marketing is another. Not much attention has been given to the form in which A3/5 might be presented, but its most

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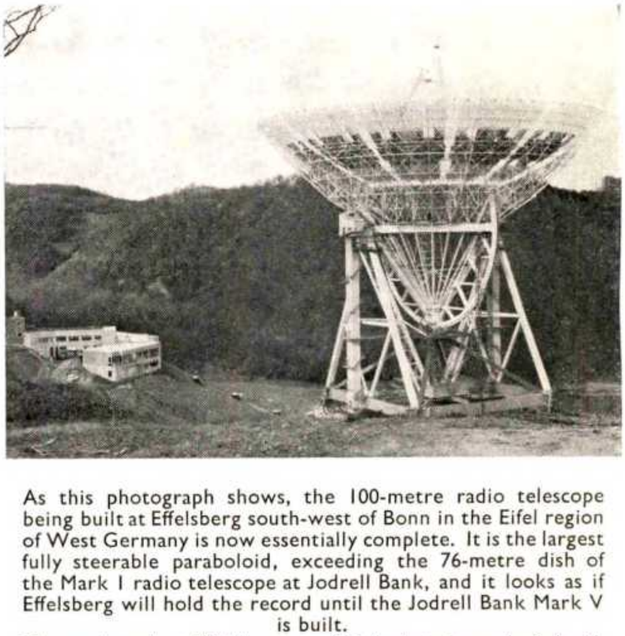
RADIO ASTRONOMY

Germany's National Dish



As this photograph shows, the 100-metre radio telescope being built at Effelsberg south-west of Bonn in the Eifel region of West Germany is now essentially complete. It is the largest fully steerable paraboloid, exceeding the 76-metre dish of the Mark I radio telescope at Jodrell Bank, and it looks as if Effelsberg will hold the record until the Jodrell Bank Mark V is built.

The steelwork at Effelsberg was finished at the end of April, and the next three months should see the final adjustment of the surface panels, the connexion of the computers and the telescope drive, and the installation of electronic equipment. The whole collecting area is designed for observations down to 5 cm and the central portion, 60 metres across, is accurate enough for work at 3 cm. Dr R. Wielebinski, director of the Max Planck Institute for Radio Astronomy at Bonn, expects preliminary observations to begin with the new telescope later this year.

likely use would be as a supplement to protein-deficient food, mixed into cereals or biscuits for people who cannot afford a balanced diet but are nevertheless suspicious of foods that are said to be good for them. Certainly, not all attempts to sell protein extracts made from soya in under-developed countries have been wholly successful.

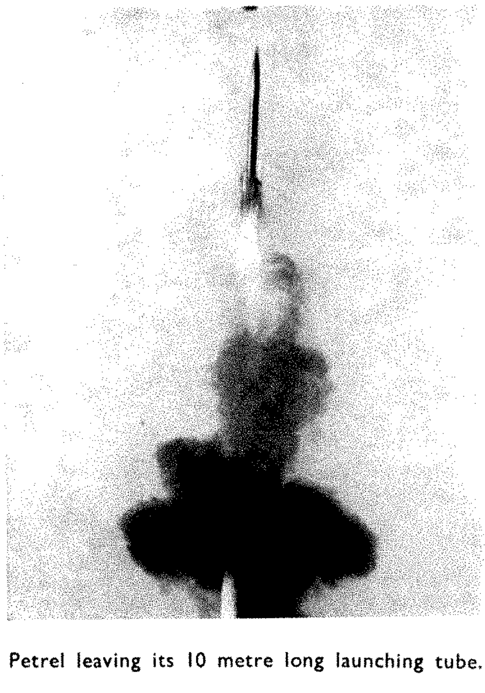
Professor Spicer, at least, is confident that A3/5 will fare better than the proteins obtained by growing yeast on petroleum hydrocarbons because A3/5 contains a better proportion of the nutritionally essential sulphur amino-acids. But the oil companies will be the first to the market: British Petroleum will be turning out yeast from its factory at Grangemouth in Scotland by the middle of the year and its French subsidiary is opening a protein plant next year. BP, however, has decided to use its product as an animal feed and the market still seems to be open for anyone who can produce proteins that humans would stomach.

ROCKETRY

Boost for Atmosphere Research

It seems almost certain that an improved version of the highly successful Petrel rocket will be developed by Bristol Aerojet Limited, with the backing of the Science Research Council. More than forty Petrel rockets have been fired since their introduction in February 1968 into the British programme of rocket investigations of the upper atmosphere. Designed as a cheap means of providing a sounding rocket chiefly for university experiments, Petrel can carry a payload of 13 kilograms to an altitude of 140 km. By adding about 15 centimetres to the 3·3 metre length of the standard version, altitudes at least 40 km higher ought to be within reach with the same 13 kilogram payload.

Although Petrel is only 19 centimetres in diameter, it seems to be big enough to carry the sort of payloads required for upper atmosphere research. In some cases the payload capability of 13 kg has been sufficiently in excess of the actual weight of the instrumentation that ballast has been carried to preserve the balance of the rocket. Thus the extra performance of the stretched Petrel is unlikely to be used to carry heavier payloads at the expense of altitude—Bristol Aerojet estimates that it could carry an extra 2 kg or so by limiting the altitude to 140 km.



Petrel leaving its 10 metre long launching tube.

Two further improved versions of Petrel are on the drawing boards at Bristol Aerojet waiting for backers. There is a Petrel 200 designed to carry a payload of 18 kg to altitudes of 220 km using three boost motors, and the Petrel Thrush to reach 350 km with a 5 kg payload. This uses the same small booster that helps to launch the standard Petrel rockets, coupled to a two stage rocket based on the Petrel as first stage.

But the order for Petrel rockets from the European Space Research Organization which Bristol Aerojet has been hoping for has not been forthcoming. Expectations were raised a year ago after the Science Research Council used the ESRO range at Kiruna, Sweden, for a series of eight Petrel launches in an investigation of the polar ionosphere. The performance of rockets in the polar atmosphere is notoriously unpredictable, and this seems to have been the first occasion when all the rockets in a Kiruna campaign have landed within the confines of the range. By all accounts the ESRO officials at Kiruna were impressed, but uncertainty about the shape of the ESRO sounding rocket programme in the future is thought to be the reason why Petrel is still used only by Britain.

EUROPEAN SPACE

ELDO's Goodbye to Woomera

The final attempt to launch a satellite from the Woomera range in the development programme of the Europa 1 rocket of the European Space Vehicle Launcher Development Organization (ELDO) will now take place on June 8. Postponed several times from the original launch date in November last year, the firing is intended to place a small Italian test satellite into an elliptical orbit of 400 km by 2,000 km. Thereafter the remaining firings in the ELDO programme will be from the French base at Kourou in French Guiana.

This will be the third orbital attempt by ELDO. Previous tries in November 1968 and July 1969 both ended in failure when the German third stage cut out prematurely. Coming directly after two test flights with dummy third stages in 1967, which were both unsuccessful because the French second stage failed to ignite, this record makes the ELDO programme the most disappointing aspect of the European space effort. Another failure shortly before the European Space Conference which is expected to meet in June to discuss European space policy would obviously be the worst impression that ELDO could make.

If the June firing is as successful as everyone hopes, a 214 kg test satellite will be launched in a direction 4·5 degrees east of north from Woomera. The prime aim of the satellite is to monitor the launch and the injection into orbit, and to make sure that the correct orbit is achieved. It will also contain an experimental package built by five members of the European Conference on Satellite Telecommunications—Belgium, Denmark, Italy, Norway and Sweden—to carry out communications tests.

The next step in the ELDO programme is the testing at Kourou of the Europa 2 launcher, which is Europa 1 fitted with a perigee stage so that a geostationary orbit can be achieved. Two firings are planned for 1971. Then everyone hopes that ELDO will be ready to carry out its first launchings for customers.

MAGAZINES

Birth of *Recherche*

A NEW French magazine has risen to join the distinguished band of scientific monthlies such as *Scientific American* and *Science Journal* which cater on a popular level for scientists as well as non-scientists. *La Recherche*, whose first issue has just appeared, is the successor to *Atomes* but aims to be more readable as well as more open to the applied and development aspects of research. The editor, M. Michel Chodkiewicz, who was also the editor of *Atomes*, describes *La Recherche* as being for "all those whose interest in scientific and technological advances is not confined to their own specialism".

With an admirably European opening, the first issue contains interviews with Professor Hans Leussink, the West German Minister of Education and Science, and Mr Anthony Wedgwood Benn, the Minister of Technology. Then follow four excellent feature articles, including a discussion of ceramic materials by R. W. Davidge of Harwell and a well illustrated essay on viruses and cancer by Luc Montagnier of the French National Scientific Research Centre.

In the last section of the magazine are brief items on French and international scientific news and longer articles on science policy and particular research topics.

Although at 7.50 francs a copy the magazine is not cheap, the indications from the first issue are that it will be good value for money. Copies may be obtained from 4 Place de l'Odeon, Paris 6^e, the subscriptions being 75 francs for France and 90 francs from abroad.

WATER RESOURCES

Filling Up the Thames

WITHIN the next ten years, underground aquifers could supply at least another 100 million gallons of water a day to the River Thames when its flow is reduced in hot, dry summers. Boreholes sunk by the Thames Conservancy in the Lambourn Valley in Berkshire have shown that it would be feasible to pump up water occasionally from the chalk and limestone formations in the Thames catchment area to supplement the flow of water into streams, such as the River Lambourn and the Winterbourne Stream, and ultimately into the River Thames. When the extra water is no longer needed, the pumps could be turned off, and the underground reservoirs would gradually fill up again during wet periods.

Two thirds of London's water and that for twenty-four other water undertakings is drawn from the River Thames. Demand is rising every year and the river's resources are already insufficient to meet the demand in dry spells without reducing the flow of water over Teddington Weir to below its statutory minimum level. There is therefore some urgency about the need for new large supplies of water for London, and the conservancy believes that it has found a solution with its pumping scheme which could be implemented at about one third to one quarter of the cost of conventional surface reservoirs and which would have very little effect on agricultural land. Analyses of the water pumped up from the chalk formations in the test area have shown it to be of the same chemical quality as the water naturally discharged to the rivers, and sampling of the fish and invertebrate fauna in the River Lambourn and Winterbourne Stream has so far shown that experimental pumping causes no adverse ecological effects. With the experience gained from its pilot scheme in Berkshire, the conservancy plans to extend the pumping scheme to other areas in the Thames catchment.

Farther downstream, there is news that the defence structure across the Thames which will protect London and Lower Thames-side from a tidal flood is to be a drop-gate barrier between Woolwich and Blackwall reaches. In a recent parliamentary reply, Mr Greenwood, Minister of Housing and Local Government, announced that the government has asked the Greater London Council to recommend a site for a barrier in that area so that work on a design can be started. The GLC has also been asked to investigate, with the London Transport Board, the need for temporary flood defences while the barrier is being built. Lord Kennet will continue to chair the policy committee which has been supervising since 1968 the studies of the GLC and other authorities into various defence schemes.

The Minister's decision gives support to the recommendations of a GLC research team which reported last January (see *Nature*, **225**, 403; 1970) that, of all the various types and location of a fixed barrage or a movable barrier across the Thames that it had investigated, it favoured a barrier in the Woolwich area, or failing that, one at Crayfordness farther downstream. A barrier of the drop-gate type at one of those two sites could operate, it was suggested, by closing at times of great risk at a point during the ebb tide, reopening on the following incoming tide unless there was a warning of a surge when it would remain shut. It was estimated that a barrier at Woolwich would take about seven years to build at a cost of about £50 million. After Mr Greenwood's announcement GLC officials said that it might be possible to combine the Woolwich barrier with another road bridge across the Thames.

RESEARCH ASSOCIATIONS

A Non-Ferrous Jubilee

THE uncertainty between now and the British election on June 18 will place a moratorium on the debate about the present government's plans for the reorganization of the research laboratories of the Ministry of Technology (see p. 679). But if the proposed British Research and Development Corporation is eventually established, then it will be in direct competition with the research associations for scarce contract work from industry. One research association which believes it is in good shape for the battle is the British Non-Ferrous Metals Research Association, which this week is celebrating its first fifty years.

Set up in Birmingham by the Department of Scientific and Industrial Research, the research association is now fast outgrowing its present site near Euston Station, London. Last year's income was £700,000, almost double what it was five years previously in 1965, and it supports a staff of 195. Most of the increase has come from contracts and for projects supported by small groups of members, such as work which the association is doing on continuous casting and on hydrostatic extrusion. By contrast the income from membership subscriptions has risen less than dramatically, last year being still slightly less than twice what it was in 1961.

This seems to be because the British Non-Ferrous Metals Research Association has by now collected all the members it can in Britain. Turning from this saturated market, the research association has recently been looking abroad for members and at present about a quarter of the income is attributable to overseas contributions. This is an area of support which the director of the association, Dr A. J. Kennedy, clearly intends to foster.

Dr Kennedy sees the future of the association as lying more in large scale projects, each financed by only a few of the members. In particular, the interests of the research association have tended to move toward the engineering aspects of metallurgical plants. But the intention is to stay at the Euston site while looking for a place to carry on the large-scale work. The continuous casting experiments are located at Widnes and the hydrostatic extrusion at Enfield, for example.

CONSERVATION

Rape of Armenia

by our Soviet Correspondent

THE importance of conservation and particularly of land conservation was grasped relatively late by the Soviet Union, largely because of its vast expanses and great natural resources. Consequently, from time to time, conditions in individual republics and regions can reach and pass the danger level without, apparently, attracting the immediate attention they merit.

A case in point is a recent disturbing report from Armenia (*Pravda*, May 14, 1970). Here, it would seem, the widespread programme of irrigation and soil improvement has lost much of its impact because of erosion, particularly in the mountain regions. The figures for these regions are disturbing; the estimated annual loss to collective and state farms is 40–80 per cent of the harvest as well as 12 million tonnes of productive soil. More than 90,000 hectares of virgin forest have been felled (presumably during the whole period of Soviet rule), and recent reafforestation schemes have so far replaced only 3,000 hectares. Anti-erosion measures are far behind schedule; over the last two years, only a third of the new anti-erosion irrigation schemes have been completed and less than half of the planned terracing of steep slopes. Likewise, schemes to turn over non-arable land to stock-rearing and to bring waste land under hay crops have fallen far short of target. Although long-term grass cultivation has been recommended as a highly effective anti-erosion measure, the area of land under grass still continues to fall rapidly. More and more irrigation schemes are being introduced (the best means of soil conservation—particularly in the geological conditions of Armenia), but are not being used to their full capacity—the case is cited of one particular installation, designed to serve 12,000 hectares which, in fact, is irrigating only some 7,000 hectares. Finally, since some of the best agricultural land in the republic, including market gardens, vineyards and well-irrigated arable land, lies close to urban centres of population, the expansion of these centres outwards is causing another type of "erosion". The new developments at Erevan are particularly cited in this respect.

Some two years ago the Presidium of the Supreme Soviet of Armenia passed a special resolution by which all land suitable for agricultural purposes and at present used otherwise or not in use should be restored to agriculture. This, the latest report notes, has only "slightly" been implemented, and recultivation has been carried out only in "insignificant areas".

The closing paragraphs of the report are illuminating. No one, it stresses, can legally be called to account for soil erosion and land loss despite the effect on the material wealth of the republic. The concept of responsibility is fundamental to the thought of the Soviet planners; should plans fail to be carried out in their entirety, somebody is found, at some level of administration, to have been responsible, whether culpably or through negligence. The disclaiming of responsibility implied in the report is evidence both of the late entry of the concept of conservation into Soviet planning and also of the even later impact of the idea into the consciousness of the average citizen, whom for fifty years the publicists have exhorted to further and further efforts of construction. Now that the emphasis is shifting, a whole new method of approach seems necessary.

POWER SUPPLY

Cool Cables Getting Hotter

A NATIONAL grid based on superconducting cables carrying direct current may sound like a pipe dream today, but the Central Electricity Research Laboratory is confidently predicting that, given ten—perhaps fifteen—years, the expected rise in power demand will strengthen the economic argument for such cables. The attention being lavished on the technical problems of superconducting cabling was evident at the CERL's open days which were held last week at Leatherhead in Surrey.

Research on superconducting transmission systems may seem foolish in view of what happened to the magnetohydrodynamic research project a few years ago. But there are some important differences. Superconductivity is, after all, a rapidly developing technology. Demand for power is continuing to double every ten years, and the case for superconducting cables grows with each additional megawatt that has to be channelled down a transmission line. At the present rate of expansion a simple extrapolation suggests that cables carrying up to 10,000 MW will be needed by the end of the decade, and superconducting cables start to become economically viable at this sort of load.

The choice between a.c. and d.c. superconducting cable links is a fine one and CERL is not yet committed to either form. The lack of inductance in a d.c. cable and the attendant lack of restriction in the shape of the cable has to be weighed against the need for a.c. to d.c. converters at the terminals. The development of solid state converters, however, has done much to simplify this problem. Cables to carry a.c., on the other hand, have to be built in annular sections, consisting of an outer surface superconductor (niobium at CERL), a region of bulk superconductor (niobium-titanium alloy) and a layer of normal metal to short the cable should any section of superconductor heat up and become resistive. The metallurgy of forming this sort of cable is still a major preoccupation at CERL, and there is no doubt that engineers are leaning steadily towards the d.c. system should the enormous cost of cooling to 4 K turn out to be justified by events.

But CERL is not gambling exclusively on sending high loads under the ground. Ultrahigh voltage overhead cables operating at around a million volts are being studied at CERL in conjunction with sister laboratories in France and Italy. CERL is concentrating on the problems of insulation, where its expertise is greatest, and has recently spent a quarter of a million pounds on an extension to its high voltage line near Brighton. Two hazards being studied are sudden surges of power and leakage due to moist conditions. The French and Italian laboratories are looking into questions of power and conductor design.

CERL is also investigating the possibility of taking voltages significantly above the present 400 kV under the ground in normal cables insulated with sulphur hexafluoride gas. The Japanese power authorities have recently completed work on an alternative underground cable using plastic insulation, which will be used to feed power into Tokyo.

SOUND

Physics ma non Troppo

A GRANT of £15,000 has been awarded by the Leverhulme Trust fund for an interdisciplinary project in physics and music at University College, Cardiff. The chief beneficiary is Mr K. Winters, who has the rare qualification of first class honours degrees in both physics and music. He will participate in a teaching programme to include courses in electronic and computer music at both undergraduate and postgraduate level and will carry out research into the capacity of computers to generate and manipulate sounds for musical purposes and for testing the mechanism of hearing itself.

Although musicians must assimilate some knowledge of the physics of sound during their training, physicists are rarely graced with a reciprocal obligation. It is pointed out at Cardiff, however, that the interface between physics and music is of direct relevance to many practical problems, including the psychological effects of hearing, the production of computer generated sounds and the analysis of other existing sound patterns. Apart from work at Cardiff, in which five research workers are involved, there are also teams working on similar problems at Princeton University and Bell Telephone Laboratories in the United States.

Parliament in Britain

Nuclear Powered Merchant Ships

THE Ministry of Technology has decided not to go ahead with the development of a nuclear powered merchant ship. Mr Harold Lever, the Paymaster General, said in reply to a question from Mr Edwin Brooks that a study group has examined the economics of nuclear powered vessels, both on a single ship and on a fleet basis, and has concluded in a report to the Minister of Technology that these ships will not be competitive with conventional vessels, at least in the foreseeable future. The report also suggests that the development of nuclear powered vessels would not help short-term employment prospects in the shipbuilding industry. (Written answers, May 15.)

Marine Pollution

THE government intends to ratify the International Convention on Oil Pollution Damage which was signed in Brussels in November last year. Announcing this intention, Mr Goronwy Roberts, Minister of State, Board of Trade, said that the convention will considerably improve the position of the claimant in the event of marine pollution damage, and he hopes that other countries will follow Britain's lead. The necessary legislation, he said, will be prepared as soon as possible.

In reply to an earlier question, Mr James Hoy, Joint Parliamentary Secretary to the Ministry of Agriculture, said that the government has allowed some industrial firms to dump polychlorinated hydrocarbon residues in sealed containers in very deep waters. All applications, he said, are very carefully examined to ensure that the disposal arrangements will not cause human or other biological hazards. Mr Tam Dalyell had asked what was the policy of the Ministry of Agriculture, in view of the established effect of chlorinated hydrocarbon residues on marine life. (Written answers, May 13.)

Adult Education

MR WILLIAM ROSS, Secretary of State for Scotland, announced that he has set up a committee under the chairmanship of Professor K. J. W. Alexander of the University of Strathclyde, to look into the provision of adult education in Scotland. The terms of reference of the committee are to consider the aims of adult courses which are educational but not strictly vocational, and, bearing in mind the need to use resources more efficiently, to make appropriate recommendations. (Written answers, May 15.)

Polytechnics

THE desirability of siting polytechnics close to other institutions of higher education is not one of the factors taken into account in choosing the centres to be developed as polytechnics. The initial location of the polytechnics is governed by the location of their constituent colleges, said Mr Gerald Fowler, Minister of State, Department of Education and Science, and although it is best to develop them on a single site, all the local circumstances, particularly the need to make proper use of existing plant, must be taken into account. It is government policy, however, that strong links should be established between polytechnics and the universities, Mr Fowler pointed out, and many polytechnics are being established in towns where universities already exist. (Written answers, May 11.)

NEWS AND VIEWS

Doing without DNA Polymerase

THE quest for a system in which to study the synthesis of DNA *in vitro* seems to have been fulfilled at last. Such synthesis has hitherto been achieved only by the much maligned enzyme DNA polymerase, but in this issue of *Nature* are the first reports of cell-free extracts which seem to lack this enzyme but can nevertheless synthesize DNA. Although there have been doubts for some time as to whether DNA polymerase is responsible for replication *in vivo*, the readiness with which it sets about polymerizing the precursors of DNA, once provided with a DNA template, has hindered attempts to characterize other enzyme systems which may synthesize DNA.

The notion that DNA polymerase does not replicate DNA *in vivo* is supported by the behaviour of a mutant of *E. coli* which lacks DNA polymerase activity when assayed *in vitro*. In spite of its defect, this strain grows well and replicates its DNA in the usual manner. On page 713 Knippers and Strätling describe how they have taken advantage of this mutant to assay cell-free extracts for other replicating enzyme activities. Another way to eliminate the unwanted activity of DNA polymerase is provided by Smith, Schaller and Bonhoeffer on page 711, who report the development of an *in vitro* assay system from which this enzyme can be washed out.

Smith *et al.* embed *E. coli* cells in a matrix of agar, fragment the agar, transform the cells into a fragile form (spheroplasts) within the agar fragments, and lyse these by osmotic shock. DNA polymerase and other soluble components can be washed out of the system to leave just fragments of membrane with DNA attached to them. When incubated with deoxynucleoside triphosphates, DNA is synthesized in a semi-conservative manner at a rate of some $1{\cdot}3\times10^3$ nucleotides per second. This synthesis, however, continues for only one to two minutes before ceasing abruptly.

Knippers and Stratling use sucrose gradients to isolate a membrane-DNA fraction from *E. coli* cells infected with phage ϕX174. When supplied with the appropriate substrates, the phage DNA in this fraction replicated semi-conservatively, again at a fast rate, and again ceasing after only a short period (five to ten minutes in this system). By using the *E. coli* mutant which lacks DNA polymerase activity, they are able to suggest that their membrane fraction may contain another enzyme apparatus which can replicate DNA.

These systems mark the nearest approach so far *in vitro* to the form of replication which prevails *in vivo*. The attempts of DNA polymerase to reproduce double stranded DNA *in vitro* proceed at a rate some two orders of magnitude below the *in vivo* rate and produce an abnormal product with strange contortions of the DNA duplex. The membrane replication systems, by contrast, demonstrate semi-conservative replication at a rate comparable with that *in vivo*. And that replication can be achieved by a DNA-membrane fraction is in line with recent theories that there is a link between the membrane and the DNA replicating apparatus of the cell.

A flaw of each system is that replication proceeds for so short a time that it cannot be complete. There is no obvious reason for this—possibilities such as precursor exhaustion have been excluded—but it will be necessary to demonstrate that the whole chromosome can be replicated if sceptics are to be convinced that this system really is that which works *in vivo*.

There remains, of course, the possibility that it is, after all, DNA polymerase which catalyses the synthesis of DNA observed in the membrane systems. It may be that this enzyme is properly active only when associated in some way with the cell membrane, so that its behaviour in other extracts is uncharacteristic. The DNA polymerase mutant may have an enzyme which is altered so that it cannot be assayed alone *in vitro*, but which is active in association with the membrane. These doubts can only be resolved by purification of the membrane systems. This may prove difficult, however, if it turns out that they function only as a complete replicating complex and cannot be broken down into individual enzyme activities.

The physico-chemical demands of the membrane-replication systems are rather different from those of DNA polymerase, and this suggests that it is more likely that they contain some other enzyme. If this is so, what part does DNA polymerase play in DNA metabolism ? This enzyme has a wide range of synthetic and degradative catalytic activities which enable it to undertake almost any action except, unfortunately, the proper replication of DNA. This versatility left it much as an enzyme in search of a function (*in vivo*, that is) until the recent demonstration that *in vitro* it can excise pyrimidine dimers from DNA. These dimers are produced in DNA by ultraviolet irradiation and distort the structure of the DNA double helix. Its ability to excise them suggests that DNA polymerase may be a repair enzyme, that is, it may maintain the correct structure of the DNA duplex by removing mismatched regions.

The DNA polymerase mutant of *E. coli* is unusually sensitive to ultraviolet light, as would be expected were DNA polymerase concerned with the repair of the damage wrought by this irradiation. On page 708, Boyle, Paterson and Setlow report the reason for this sensitivity. They find that the mutant is not defective in its ability to excise thymine dimers. But it does have an abnormally active exonuclease activity which extensively degrades the DNA of mutant cells after ultraviolet irradiation. If this is a result of the mutation to DNA polymerase, it may be that the mutant enzyme is no longer able to synthesize DNA *in vitro*, but retains an ability to degrade DNA *in vivo*, although with altered characteristics.

Trgovčevic and Kucan suggest (page 752) that DNA polymerase may be concerned with the degradation of DNA *in vivo*. Irradiation with X-rays or gamma-rays introduces internucleotide chain breaks into the DNA of *E. coli*, and after this the bacterial DNA is degraded quite considerably. They find that the products of degradation induced by gamma-rays are such as to result from an enzyme action which is exercised in *E. coli* only by DNA polymerase. This invites another experiment with the DNA polymerase mutant; it would be interesting to see whether the characteristics of this degradation are affected by the mutation. It seems that definition of which of its many postulated roles DNA polymerase actually fulfils *in vivo* must await a more complete investigation of the precise nature of the polymerase mutation.

ENZYMES

Counting Sites

from our Molecular Biology Correspondent

ENZYMES of which the molecular weights and number of subunits have been in dispute are legion. Particular frustration and bad temper seem to result when the number of subunits determined by molecular weight measurements disagrees with the apparent number of active sites. An interesting case has now been resolved by Mann *et al.* (*Biochemistry*, **9**, 1348; 1970). This is human transferrin, an iron-binding protein present in serum. It is accepted that the molecule has two iron-binding sites with identical characteristics. Reported molecular weights vary between 68,000 and 93,000, and there has also been a choice between the views that the protein consists of a single chain, and that it can be caused to dissociate into two subunits.

The new results give a molecular weight by sedimentation equilibrium of 77,000, which agrees with two out of the range of earlier measurements. The fully denatured, reduced protein examined in guanidine hydrochloride solution has a similar molecular weight, and two other methods involving complete denaturation and dissociation of subunits likewise lead to a value in the 70–80,000 range. It thus appears that transferrin represents an unusual case of a single polypeptide chain possessing two identical functional sites. In terms of precedent this is so unexpected that one is immediately forced to consider whether the protein may nevertheless in reality not consist of two subunits, which are covalently fused. Mann *et al.* prepared tryptic fingerprints, and counted the number of peptides. Subjective as this procedure is apt to be, it still seems that there are altogether too few fragments for the number of trypsin-labile bonds (one for each lysine and arginine) expected on the basis of amino-acid composition. Thus some fifty-four spots can be discerned, whereas seventy-six are expected, and specific staining for tyrosine, tryptophan and histidine indicated that peptides containing these residues were also present in only half to two-thirds of the expected number. The evidence is circumstantial, for with such a large number of fragments there can be no assurance. that all are resolved, but the results are at least consistent with the conjecture hazarded by Mann *et al.* that transferrin may indeed consist of two identical polypeptide segments that have insome way grown together It may be recalled that covalent association between two subunits of β-galactosidase in wild-type *E. coli* was postulated recently by Goldberg (*J. Mol. Biol.*, **46**, 441; 1969), the covalent link between them being reportedly absent in a mutant.

A protein long the centre of bitter wrangles is myosin. It is now established that it consists of two principal chains and some additional small associated chains. If any lingering doubts remained about the presence of two active sites in myosin, Murphy and Morales (*Biochemistry*, **9**, 1528; 1970) have dispelled them still further: using an ATP derivative with a built-in chromophore that absorbs in the near-ultraviolet, they measure the number of binding sites from the magnitude of the transient absorbance change that accompanies the introduction of the substrate into the protein solution, and find a value of close to 2.

But the greater interest in this work is that the new chromophore contains a thiol group, which, when the *p*H is raised above 8, will react with a cysteine side chain at or near the active site. The enzymatic activity is then inhibited, and is regenerated only when the disulphide bond which has been formed is broken by addition of excess of an added thiol. Two moles of the chromophore are then again released per mole of myosin. When the light chains of the labelled myosin are stripped off, the label comes with them. This is an agreeable result, for it strongly supports earlier suggestions that a low molecular weight species on the myosin carries or at least closely associates with the ATPase sites.

NUCLEIC ACIDS

Searching for Messengers

from our Cell Biology Correspondent

IDENTIFYING messenger RNAs or their precursors in mammalian cell nuclei is proving as hard as picking out a handful of needles from a haystack. The chief problem is that mammalian nuclei contain a heterogeneous collection of high molecular weight RNAs which have some of the characters of messengers. Apart from their large size and heterogeneity these RNAs undergo rapid turnover in the nucleus; apparently the molecules never migrate to the cytoplasm and never participate in protein synthesis. As a result, in pulse labelling experiments the heterogeneous nuclear RNAs form an effective screen obscuring the detection of the messengers.

A few weeks ago (*Nature*, **226**, 215; 1970) I mentioned the experiments of Darnell and his collaborators which are at least compatible with the idea that in HeLa cells a small fraction of the total heterogeneous nuclear RNAs are precursors of cytoplasmic mRNAs which escape destruction in the nucleus and undergo maturation by cleavage before migrating to the cytoplasm. This hypothesis, attractive if only because it suggests a function for the nuclear heterogeneous RNAs, implies that the nuclear precursors of mRNAs are considerably larger than the mRNAs to which they give rise. Unwanted sequences must therefore be chopped from the molecule as it matures, just as the precursor of ribosomal RNA is cleaved to yield the mature RNA.

Clearly the best way to test this suggestion is to look for the precursor of a particular mRNA, but in mammalian cells that is almost impossible. Lindberg and Darnell (*Proc. US Nat. Acad. Sci.*, **65**, 1089; 1970) have therefore turned to mouse 3T3 cells transformed by SV40 virus which have the viral genome stably incorporated and make SV40 specific RNAs. The amount of viral specific RNA in the nucleus and cytoplasm of the transformed cells is very small but at least it can be assayed.

Using pulse labelling and DNA/RNA hybridization, Lindberg and Darnell find that the nuclear RNA which hybridizes with SV40 DNA is larger (molecular weights range from $2\text{-}4\times10^6$ daltons) than the cytoplasmic RNA which hybridizes (molecular weight about $1{\cdot}7\times10^6$). Of course, experiments of this sort do not prove that large nuclear molecules are precursors of the small cytoplasmic molecules but at least they do not preclude that possibility. The other interesting point to emerge is that the largest nuclear RNAs containing SV40 sequences are larger than the SV40 genome. Either these RNAs contain some cellular as well as viral sequences or they may be the products of transcription of at least two SV40 genomes in tandem. This latter alternative is feasible because cells transformed with SV40 are believed to contain some twenty to sixty copies of the virus, which may all be in tandem.

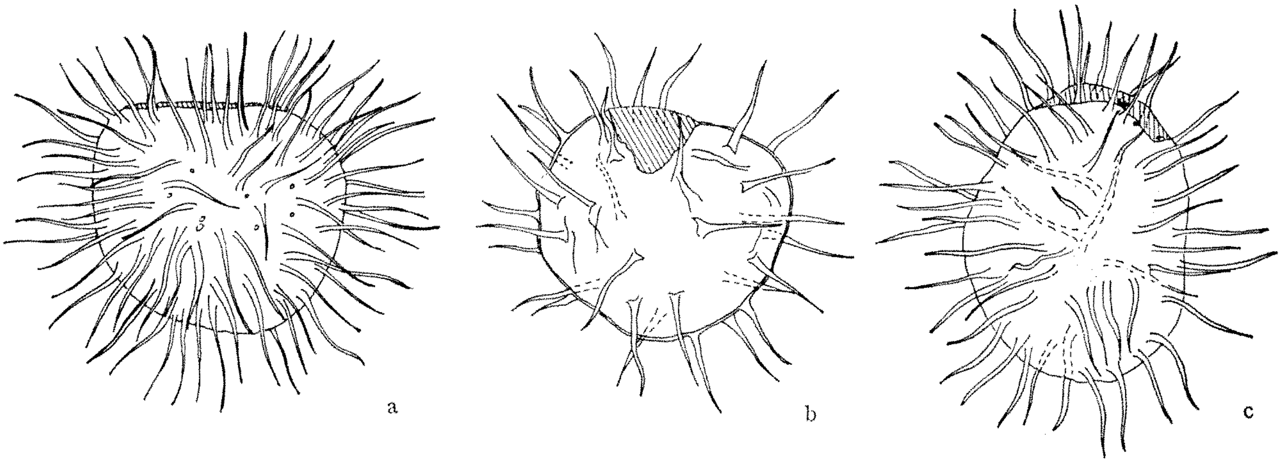
It will be particularly interesting if Darnell's group finds the first alternative to be correct, but even if all the sequences in the largest nuclear RNAs prove to be viral that will strengthen the case for asserting that viral genomes can occur in tandem in transformed cells.

The synthesis of SV40 mRNA in transformed 3T3 cells takes place against a background of synthesis of 3T3 mRNA. Cheevers and Sheinin (*Biochim. Biophys. Acta*, **204**, 449; 1970) have recently analysed the metabolic stability of messengers associated with polysomes in these cells. Using very low concentrations of actinomycin *D* to block synthesis of ribosomal RNA while leaving mRNA synthesis unaffected, they find the kinetics of incorporation of uridine indicate that the cells contain at least two classes of mRNA. One is predominant but ephemeral, with a lifetime of about 2 h, while the other is stable for at least 6 h. The significance of these differences for the regulation of gene expression is, needless to say, obscure.

MICROPALAEONTOLOGY

Finds from the Clay

Two new genera and twelve new species of fossil dinoflagellates have been yielded by exposures of Kimmeridge Clay in England, Scotland and France. They have been described by G. V. Gitmez together with many other species of these unicellular organisms, living examples of which botanists and zoologists claim as both algae and protozoa because of their apparently intermediate characteristics (*Bull. Brit. Mus.* (*Nat. Hist.*), **18**, No. 7; 1970). All that remains of the Upper Jurassic species preserved in the Kimmeridge Clay are the hard outer coverings produced by the soft bodies. The variety of form among these structures provides palaeontologists with scope for a great deal of taxonomic activity, such as the description of *Cleistosphaeridium polyacanthum* (*a* in the illustration, ×1,400). This new species was found at Ringstead Bay in Dorset, and Gitmez says that it is morphologically intermediate between *C. ehrenbergi* (*b*, ×1,280) and *C. polytrichum* (*c*, ×1,280).



NATURAL HISTORY

Running a Museum

The work of the modern natural history museum is, according to Dr Ernst Mayr, more relevant to today's environmental problems than most of the work carried out in experimental laboratories. Writing in his last annual report as director of the Museum of Comparative Zoology at Harvard University, Mayr emphasizes the need for interdisciplinary research and teaching, such as he has been encouraging at Harvard for the past eight years. All aspects of the living animal and its evolution come into the museum's scope—ecology, behaviour, anatomy, physiology, biochemistry and genetics, as well as the more traditional museum activities of taxonomy and the maintenance of collections. Field expeditions, of course, still play an important part in the museum's life, and staff and students ventured far and wide in 1969, returning the richer for knowing more about the evolution of early hominids in East Africa, the chromosome patterns of the iguanid lizard, *Sceloporus*, in Mexico, the adaptive radiation of penguins, and the coadaptations of fruit eating birds and the plants they eat in Costa Rica.

Many of the taxonomic studies in the museum have important implications outside their own specialized fields. Dr R. W. Matthews, for example, has been revising a little-known genus of wasps called *Spathius* that parasitizes bark beetles, important economic pests of conifers. In the past, it has been difficult to identify these wasps accurately, but Matthews has been able to work out keys for their identification and has recorded the host range of each species. It seems that some of the wasps attack several different species of bark beetle and other wood infesting insects, and rather than being host specific they are influenced by the type of tree which the beetles attack. This research may provide some clues for biological control of the beetles.

Correct classification of animals and plants is essential to comparative studies such as those carried out at Harvard, so that it is important that the basic unit of classification, the species, is well defined and delimited. With all the sophisticated techniques at the fingertips of taxonomists today, it might be assumed that many previous classifications, some worked out well over a hundred years ago, would now be found to be faulty. This certainly does not seem to be the case for some groups. Mayr, in collaboration with Dr L. Short, of the American Museum of Natural History in New York, has found, for example, that there is little disagreement today about the 606 species of North American birds, even though most were first described in the eighteenth century. For molluscs, however, the story is rather different. Recent estimates of the number of living species of these animals range from 40,000 to 150,000, the most recent being 107,000. The reliability of these figures has been assessed by Dr K. J. Boss of the museum, who has analysed the most recent and thorough revisions of families and genera. He has found that each real species has been named at least five different times, with some freshwater and land forms being named two hundred times. Analyses of the faunal distribution of molluscs have confirmed that the total number of species is far smaller than is usually claimed, and Boss puts his estimate at about 50,000.

SEA FOODS

Prawn Farms on the Way

THE rearing of prawns on a commercial scale has become a possibility now that they have been successfully cultured from larval to adult stages at the Ministry of Agriculture, Fisheries and Food Experiment Station, Conway, Wales. J. R. M. Forster reports encouraging results of the first outdoor rearing trials which demonstrate the feasibility of using, on a larger scale, the experimental techniques worked out at Conway (*Fish. Invest.*, Series II, XXVI, No. 6; 1970).

Palaemon serratus, the prawn in question, had already been reared to the first juvenile stage (M. R. Reeve, *Fish Invest.*, Series II, XXVII; 1969), and Forster's task was to provide suitable culture conditions in which the various juvenile stages could moult and grow to adulthood. Experiments began in the laboratory with prawns in beakers and plastic buckets of aerated seawater, and later moved outside where tanks and plastic dustbins were brought into use.

Food requirements proved a problem. Newly metamorphosed juveniles fared best on live daily rations of the fairy shrimp *Artemia*, later being weaned on to prepared food consisting of chopped mussel, crab, shrimp and lugworm. After weaning, the young prawns grew best when given mixtures of prepared foods rather than a diet of pure mussel or shrimp, for which Forster can offer no explanation. Later juveniles, from about two weeks after metamorphosis, were less demanding, growing just as well on a single food as on mixtures.

The other great problem was cannibalism, which was the largest single cause of mortality in laboratory populations of juvenile prawns. Many freshly moulted, soft individuals were attacked and eaten by their hard-shelled companions. The prey could not be induced to hide in lengths of tubing, and so their fate depended only on their ability to escape from the predators. Cannibalism turned out to be related directly to population density, and there were good signs that increasing the floor space, in this case by placing horizontal slats in the tanks, decreased cannibalism.

The optimum temperature for survival and growth of prawns in culture seems to be between 20° and 24° C; at 18° C growth was generally retarded, although survival was good, and at 26° C growth was good but survival poor. Forster points out that this is a significant finding if commercial rearing of prawns is to be attempted in Britain, where temperatures in outdoor tanks reach 20° C only from June to early September. At other times of the year artificial heating would be necessary.

As far as light conditions are concerned, growth was best in total darkness but survival was reduced. There seems to be no advantage in continuous illumination at any of the intensities that have been tested, although it is apparently possible that continuous illumination below six lumens per square foot results in improved growth. The prawns did not seem to be affected by the turbidity of the water as long as it was aerated vigorously to maintain the concentration of dissolved oxygen.

Forster thinks that the techniques now developed for culturing prawns would probably be best used on a commercial scale for some species other than *Palaemon serratus*, which has some inherent handicaps, notably its failure to survive for more than a few days at temperatures below 4° C, and the small size of the males, which, at a maximum of 5 grams, do not reach an optimum commercial size. Although it may prove possible to solve the temperature problem by making use of warm water effluents from power stations, Forster feels that it would be worth looking for another species to culture in Britain.

ELECTRON MICROSCOPY

More Scope for Scanning

from a Correspondent

THE range of applications of scanning electron microscopy continues to grow fast, as delegates to a symposium on this topic learnt at the Illinois Institute of Technology in Chicago from April 28 to 30. Most contributions, however, dealt with applications of what can now be regarded as the conventional mode of operation of the scanning electron microscope, using secondary electrons emitted in the backwards direction to form the image. Applications in metallurgy, semiconductor electronics, materials science, biology and medicine are expanding at a great pace, but little of the work involved makes any general contribution to the method. How many more such specialist conferences will be justified before the method is regarded as established ?

As usual, the efforts of Professor Albert Crewe (University of Chicago) aroused a great deal of interest. He demonstrated a 10 Å resolution last year and claims something like 5 Å this year, in a transmission scanning electron microscope with a field emission source (described earlier in *Nature*, **226**, 213; 1970). This method has the advantage over conventional transmission electron microscopy that energy loss analysis can be conducted on the transmitted beam and that it is sensitive to differences in atomic number. It is possible to examine much thicker specimens than are used in a transmission electron microscope run at the same accelerating voltage, and a much higher contrast can be obtained with the Crewe method.

In the biological sphere, it was interesting to find that the value of using as low an accelerating voltage as will give the required resolution is becoming widely recognized. Mr A. N. Broers (IBM) showed that it is possible to achieve high resolution, certainly better than 100 Å, from a biological sample—in this case of bone crystals. A lively discussion followed the description by Mr W. C. Lane (Battelle Memorial Institute) of his environmental control stage which allegedly makes it possible to examine water drops in the scanning electron microscope. Even this was doubted and it was suggested that the surface of the drop might be a thin skin of ice. The usefulness of a freezing stage in the examination of frozen botanical specimens was demonstrated by Dr P. Echlin and others from the University of Cambridge.

It is surprising that, in a meeting devoted to the results of a method of microscopy with a great depth of focus, so little attention was paid to measurements made in these images. Simple magnification markers in images of rough surfaces serve practically no useful purpose and it is to be hoped that some alternative method of conveying approximate information about sizes, such as signifying the nominal width of the field, will soon be adopted.

EARTH'S MANTLE

Viscosity and Drift

from our Geomagnetism Correspondent

It is implicit in the theories of continental drift and sea floor spreading that at least the upper mantle of the Earth, though ostensibly solid, behaves in certain circumstances as a viscous fluid. The first application of viscous flow theory to the asthenosphere, in fact, owed nothing to continental drift but arose in calculations of the viscosity required to enable Fenno-Scandia to return to isostatic equilibrium after the removal of the most recent glaciation. Working along these lines, Haskell (*Physics*, **6**, 265; 1935) estimated the anelastic response of the upper mantle to applied stress in terms of a Newtonian fluid viscosity of the order of 10^{22} poises. Later, Crittenden (*J. Geophys. Res.*, **68**, 5517; 1963) derived a value of the order of 10^{21} poises from a similar study of the Lake Bonneville uplift.

These estimates were derived from application of viscous theory over comparatively localized areas of the Earth, and may or may not be valid over regions of continental or oceanic extent. The question thus arises as to whether the more modern concepts of global tectonic mobility can be used to calculate the viscosity on which they themselves depend. Cramer (*Science*, **167**, 1123; 1970), using a very simple model, has shown that they can and that, furthermore, the result turns out to be almost identical with the earlier results of Haskell and Crittenden.

The basis of Cramer's analysis is to assume that the decrease in amplitude of ocean bottom relief from the mid-Atlantic Ridge towards the continents arises from viscous creeping of the bottom features under gravitational stress. Examining an east–west profile across the mid-Atlantic Ridge at 25° N, and assuming a sea floor spreading rate of 2 cm yr^{-1}, Cramer obtained a mantle viscosity of between 1·5 and $3{\cdot}9\times10^{22}$ poises with a most probable value of $2{\cdot}1\times10^{22}$ poises. A similar analysis of a second profile at 28° 40′ S led to an apparent change in viscosity at some time in the past. Up to a point 160 km to the west of the Ridge the calculated viscosity was $4{\cdot}6\times10^{22}$ poises whereas beyond 160 km it turned out to be $8{\cdot}2\times10^{22}$ poises.

Whether this discontinuity in viscosity is real is a moot point. Clearly much depends on the accuracy of the assumed spreading rate; and an apparent change in viscosity could equally well be due to a change in spreading rate at the 160 km point. Indeed, there is already evidence that the spreading rate has decreased along some portions of the mid-Atlantic Ridge. The actual spreading rates deduced by Heirtzler *et al.* (*J. Geophys. Res.*, **73**, 2119; 1968) are 1·3 to 1·4 cm yr^{-1} at 25° N and 2·0 cm yr^{-1} at 28° 40′ S. The use of these values would tend to make the viscosity values converge, bringing them within the error of least squares analysis.

The essential result, however, is that in spite of the large difference in the horizontal dimensions of Fenno-Scandia and Lake Bonneville, on the one hand, and the Atlantic Ocean, on the other, a very simple model for the ocean floor produces values for mantle viscosity remarkably consistent with the earlier results of Haskell and Crittenden.

MAGNESIUM ISOTOPES

Arcturus Like Solar System

from our Cosmology Correspondent

By studying the magnesium hydride bands in solar and stellar spectra the relative abundances of the magnesium isotopes can be determined fairly accurately. The terrestrial and meteoritic abundance ratios for ^{24}Mg : ^{25}Mg : ^{26}Mg are known to be 80 : 10 : 10, and the solar abundance ratios were thought to be roughly comparable, until in a study of sunspot spectra D. Branch found ratios of 60 : 20 : 20 (*Astrophys. J.*, **159**, 39; 1970). Branch has joined forces recently with R. A. Bell to measure these isotope ratios for the star Arcturus, a *K* giant.

This analysis has been carried out using a computed model in which the abundance ratios, Doppler velocity, atmospheric temperature and other physical parameters of the atmosphere can be varied to produce theoretical line spectra, a boundary temperature of 3,360 K producing the best fit with the observed colours for

Arcturus. R. Griffin's recent *Photometric Atlas of the Spectrum of Arcturus* provides the required observational detail for comparison with these computed spectra, from which Bell and Branch find that the observations are consistent with ratios of 80 : 10 : 10, like those found on Earth and differing considerably from the revised values for the Sun (*Astrophys. Lett.*, **5**, 203; 1970). It is certainly clear that both 100 : 0 : 0 and 60 : 20 : 20 can be ruled out as possible ratios for the magnesium isotopes in Arcturus and this must cause some doubt as to whether Branch's earlier result can be taken as typical of the whole Sun. If the magnesium isotopes are present in the same proportions for the whole solar system and in other stars, we have a useful insight into the formation and evolution of stars and planets; the 80 : 10 : 10 ratios would certainly seem more reasonable for the Sun on the basis of theoretical nucleo-synthesis rates, so that the overall inference of these calculations must be that general conclusions based on the spectra of sunspots should be treated with great caution.

SPECTROSCOPY

Power of Photon Echoes

An ingenious way of measuring hyperfine structure has been suggested which uses what is known as the photon echo technique. This involves bombarding atoms with two pulses of light separated by a short time interval and retrieving echo frequencies of two closely spaced energy levels which can be made to beat together. A. I. Alekseyev (*Phys. Lett.*, **31**, 495; 1970) has shown that by making the frequency of the incoming light quite similar to those of the energy levels in question it is possible to resolve quite remarkably close energy levels in the atoms, and he has suggested some ways of testing this.

The important feature of the technique seems to be that the frequencies picked up in the echo are independent of the initial input frequency. This happens because of the way in which the electrons are excited and relax between the two closely spaced input pulses. The two outgoing waves then combine to produce beats with a frequency equal simply to the difference between those of the atomic energy levels in question.

Although the photon echo method has been used to study chromium ions in ruby and other similar systems Alekseyev's application seems to be a quite new suggestion. There are already, of course, a variety of techniques for measuring hyperfine energy levels including conventional microwave or radio spectroscopy. But both the precision claimed for the photon echo method and the novelty of the interactions involved lend particular interest to demonstrating these ideas in an actual experiment.

MARS

Mariner Spots Phobos

by our Astronomy Correspondent

One of the two satellites of Mars has been found on three of the more than two hundred television pictures of Mars taken by Mariners 6 and 7 last year, and on one of the pictures the image of the satellite, Phobos, is large enough to provide a valuable estimate of its size. Rather surprisingly, Bradford A. Smith of the New Mexico State University, a member of the Mariner evaluation team, reports that Phobos is decidedly elliptical in shape (*Science*, **168**, 828; 1970). Although this suggests that Phobos is asteroidal in origin, it raises once again the awkward problems in accounting for the orbit of Phobos which is equatorial and only 3,750 miles from the surface of the planet. So far there is no evidence that either of the Mariners recorded the second Martian satellite, Deimos, which is roughly the same size as Phobos but orbits at about six Mars radii from the surface. But there are a further three pictures on which Phobos has been tentatively identified, and Smith emphasizes that his analysis is based on only the preliminary processing of the Mariner television experiment.

The essential result reported by Smith is that Phobos is elongated in the direction of its orbital motion, measuring fourteen miles in diameter at its equator and eleven miles at the poles. There is no information about the third dimension, of course. Smith also estimates that Phobos has an albedo of only 0·065, making it the darkest body in the solar system. Up to now Mercury with an albedo of 0·100 has been held to have this title.

The problems which the ellipticity raises have been discussed most recently by G. A. Wilkins (*Istituto Nazionale di Alta Matematica, Symposia Mathematica*, **3**; 1970). It is difficult to see how a captured asteroid can be constrained to move in the equatorial plane like both Phobos and Deimos, yet there seems to be no reason why asteroids should be captured directly into equatorial orbits. On the other hand, if the satellites were formed *in situ* at roughly the same time as the formation of Mars, by accretion in a cloud of dust, then an equatorial orbit seems more likely. This is because particles orbiting the planet in any plane but the equatorial plane are easily dispersed. Another drawback to the capture hypothesis is that any mechanism which is strong enough to force Deimos into its present nearly circular, equatorial orbit will be so strong as to make Phobos collide with Mars within a short interval of time.

This is why Wilkins seems to favour the accretion hypothesis, but the asteroidal shape of Phobos revealed by Mariner 7 must now be a serious obstacle. There is no reason why a moon formed by accretion should be anything but spherical, but the asteroids are known to be often irregular in shape. Thus the Mariner photograph has exacerbated a problem which has been a headache in orbital dynamics for years. The discovery makes even more urgent the task of improving knowledge of the orbits of Phobos and Deimos which should add to the information about how the system has evolved, as well as saying something about the shape of Mars. One astronomer has even gone so far as to suggest the soft landing of a radio transmitter on Phobos, nicely illustrating the fixation that those dealing with orbital mechanics have on the Mars problem.

The low albedo that Smith has recorded will also require some explanation, but Smith's view is that it is because Phobos lacks the dusting of interplanetary particles which the Moon (albedo 0·115) and also feasibly Mars (0·154) and Mercury have. This is a natural outcome of the low escape velocity of a small body, and what is being seen is the dark colour underlying rock.

Nickel in Western Australia

by
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Nickel ore deposits discovered in Archaean ultramafic greenstones and Tertiary laterites have resulted in frenzied ore search operations in Western Australia. This article outlines the geological background to the discoveries.

THE discovery of nickel deposits at Kambalda[1] in January 1966 has precipitated a feverish search by the world's major mineral exploration companies which rivals the gold rush of the early 1890s. Few stones remained unturned in the million square miles of Western Australia by the turn of the century, but the prospector of those days was interested only in gold. As a result, the rich nickel deposits discovered at Kambalda were overlooked, in spite of the occurrence of nickeliferous limonitic gossans in surface pits sunk in this area soon after the discovery of gold there in 1898.

Mineralization in Western Australia[2], with one exception, is confined to the Pre-Cambrian rocks which occupy more than a quarter of a million square miles. Primary nickel deposits occur in Archaean greenstone belts and secondary deposits occur in Tertiary laterites. The geological background to the occurrence of nickel in Western Australia therefore focuses on the earliest (Archaean) and latest (Tertiary to Recent) parts of the geological record.

Archaean of Western Australia

Archaean rocks occupy approximately 300,000 square miles of Western Australia forming the Yilgarn and Pilbara Blocks, and possibly part of the Kimberley Block (Fig. 1). The geological sequence is similar in both cratons and resembles those of other Pre-Cambrian Shields, such as the Canadian, Indian and Southern African Shields[3]. These cratons consist largely of granitic gneisses with narrow older belts of metavolcanic and metasedimentary rocks and younger massive granite plutons[4].

In the Yilgarn Block, the oldest recognizable rocks are metavolcanic "greenstones" and interbedded metasedimentary "whitestones" of the Kalgoorlie–Yilgarn Succession. The Greenstone Phase of this succession consists predominantly of altered basaltic lavas, often pillow-structured indicative of submarine origin, with subordinate fragmental volcanics, and occasional thin interbedded layers of chemically deposited banded iron formation and jaspilite. The Whitestone Phase consists of clastic sediments (shales, sandstones and conglomerates) and acid volcanics. Recent work in the country between Norseman and Kalgoorlie[5,6] has established a stratigraphic succession consisting of repeated volcanic cycles, each varying from basic volcanics upwards to acid volcanics and clastic sediments. Although these greenstones and whitestones are undoubtedly the oldest rocks yet found in the Australian Shield, they have obviously been erupted on to an earlier Earth crust which has nowhere been identified with certainty.

The interbedded basalts, acid volcanics and clastic sediments of the Kalgoorlie–Yilgarn Succession have been concordantly intruded by ultrabasic magma now represented largely by serpentinites, the result of serpentinization of peridotitic rocks. These intrusive serpentinites are probably Alpine-type serpentinites belonging to a late stage in the geosynclinal development represented by the earlier basalts and sediments which were strongly folded and intruded by the serpentinites. It is these altered intrusive ultramafics which seem to be particularly significant so far as the nickel mineralization is concerned. There are also layered, gravity-differentiated, basic igneous stratiform complexes which have been less metamorphosed and are characterized by an abundance of primary unserpentinized minerals, such as olivine and pyroxene. They vary from ultramafic rocks, such as various peridotites and pyroxenites in the lower parts to mafic rocks, such as gabbros and norites, near the top[7,8]. The ultramafic parts of these intrusive stratiform complexes may also be significant so far as nickel mineralization is concerned. Indeed, these complexes are comparable, although much smaller, with the Giles Complex of Central Australia on which the Wingelinna nickel deposits developed by lateritization processes during Tertiary times.

Throughout the Yilgarn Block the Kalgoorlie–Yilgarn greenstones and whitestones and associated serpentinites and stratiform basic complexes have been isoclinally folded on NNW-trending axes and contemporaneously metamorphosed and granitized. They now occur as elongated, synformal, steeply dipping remnants in a vast

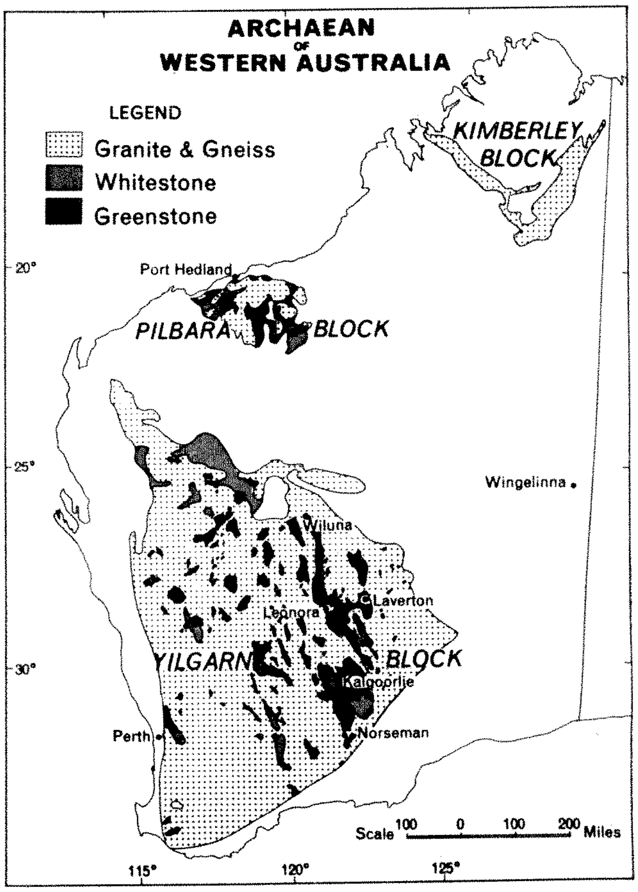

Fig. 1. Archaean blocks of Western Australia, showing "greenstone" belts.

expanse of granitic gneiss, which has resulted from granite intrusion and granitization of the Kalgoorlie–Yilgarn Succession (as evidenced by relict stratigraphy in the form of lenses of resistant beds, such as jaspilite). The gneissic foliation of the granitic gneisses conforms to the structure of the folded and metamorphosed greenstones and whitestones. The metamorphic grade of the Kalgoorlie–Yilgarn rocks seems to be a function of their proximity to the enveloping granitic gneiss[9]. Thus, at Kalgoorlie, which is centrally placed in the largest of these Kalgoorlie–Yilgarn remnants, the rocks have been subjected to greenschist facies metamorphism and retain much of their original structure and texture, the basalts being uralitized or chloritized, the ultramafics (peridotites and related rocks) being serpentinized, and the sediments not metamorphosed sufficiently to obscure their original clastic textures. In the marginal parts of the greenstone–whitestone inliers and in narrow greenstone belts the original basalts have been more highly metamorphosed to plagioclase–hornblende schists, the ultramafics to talc–tremolite and tremolite–chlorite schists, and the sediments to micaceous schists and quartzites.

The folded Kalgoorlie–Yilgarn Succession was intruded by discordant granites in late-Archaean times (about 2,615 million years ago). These granites, which are either homogeneous and medium to fine grained or coarse porphyritic and flow-banded in the marginal parts, tend to occur in structurally controlled elongated batholiths, elongated in a general north–south direction.

All the previously mentioned Archaean rocks in the country between Laverton and Norseman are intruded by east–west basic dykes (the Widgiemooltha Dyke Suite), the largest of which can be traced for 200 km and varies in size up to 3 km wide. These dykes have been radiogenically aged at 2,420 million years. Like the stratiform layered intrusions, they are also magmatically differentiated into ultramafic pyroxenites and mafic norites, the ultramafics of which may be of interest so far as the occurrence of nickel is concerned.

The Archaean of the Pilbara Block[2] resembles that of the Yilgarn Block in all but structure. It consists of folded metamorphosed greenstones and associated metajaspilites of the Warrawoona Succession and overlying sediments of the Mosquito Creek Succession, all intruded by a granitic complex. The style of folding differs from the NNW-trending structure of the Yilgarn Block insofar as there is no consistent trend, the tightly folded Warrawoona–Mosquito Creek rocks seeming to wrap around the larger granitic masses.

Archaean Nickel Deposits

The nickel finds made so far (except the lateritic nickel deposits of Wingelinna) are confined to the Archaean greenstones of the Norseman–Laverton–Wiluna Belt (Fig. 2). Other than in company reports, published information is available only about the initial discovery at Kambalda[1], 35 miles south of Kalgoorlie. Kambalda is situated in the same greenstone belt as Kalgoorlie, where the stratigraphic succession of a conformable sequence is as shown in Table 1. The Hannans Lake Serpentinite, an unlayered homogeneous intrusive outcropping south of Kalgoorlie, is a dark green massive serpentinite derived from a peridotite fine to medium grained olivine with interstitial pyroxene. It is interesting since the nickel sulphide ore bodies at Kambalda occur in a similar rock. But because of the intense isoclinal folding and accompanying strike faulting, along with paucity of outcrop, the relationship between the Hannans Lake Serpentinite and the Kambalda ultramafic has not yet been established.

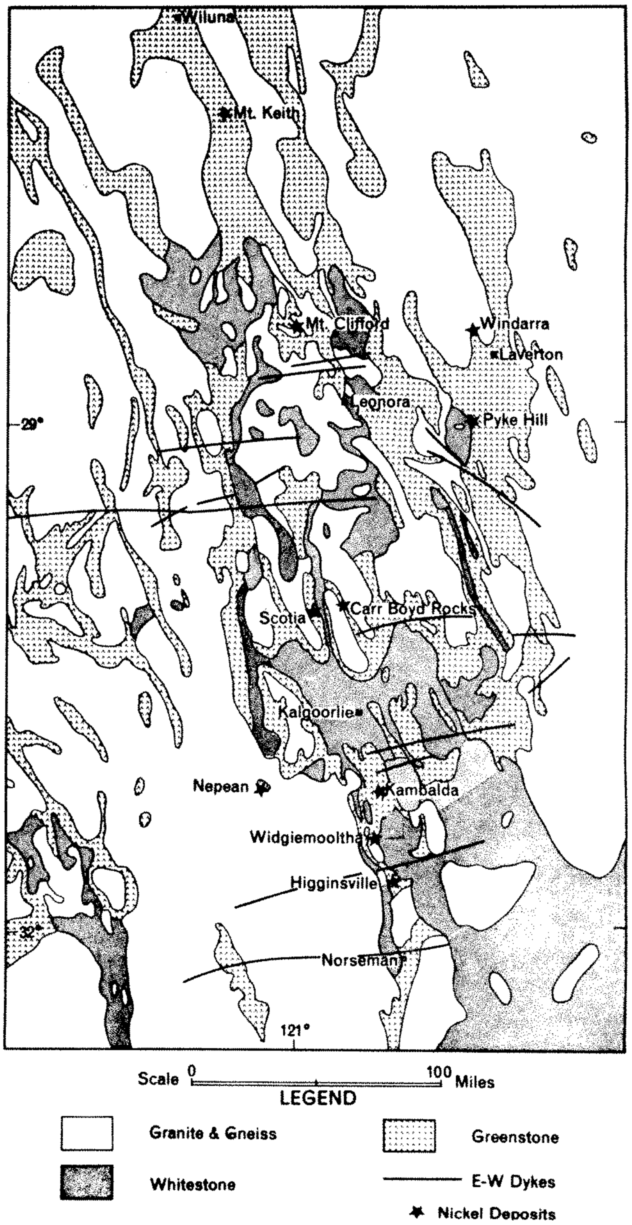

Fig. 2. Nickel deposits of the Norseman–Laverton–Wiluna "greenstone" belt (geology after Geological Survey of Western Australia, 1966).

The Kambalda nickel sulphide ore bodies are associated with a sill-like ultramafic body which lies more or less conformably between two metabasalts which contain some pillow structures. The ultramafic is a layered intrusion consisting essentially of varying amounts of serpen-

Table 1. STRATIGRAPHIC SUCCESSION AT KALGOORLIE (AFTER WOODALL[10])

Rock unit	Thickness (feet)	Lithology
Black Flag Beds	10,000	Acid to intermediate lavas and pyroclastics, overlain by sandstone, shale, slate and quartzite
Golden Mile Dolerite	1,000–3,000	Sill of meta-quartz dolerite
Paringa Basalt	1,000–3,000	Metabasalt (in part, pillow lavas)
Williamstown Dolerite	500–1,000	Sill of metadolerite transitional to meta-quartz dolerite near top and hornblendite [= metapyroxenite] near base
Kapai Slate	10	Graphitic slate
Devon Consols Basalt	200– 500	Metabasalt (pillow lavas)
Hannans Lake Serpentinite	1,000–3,000	Massive serpentinite

tine, talc, chlorite, amphibole and carbonate. Shale and banded chert present in the succession are prominent on the upper and lower surfaces of the ultramafic, and discontinuous but conformable chert bands, up to 50 feet thick, also occur within the ultramafic, indicating possible successive intrusions of ultrabasic magma. It is noteworthy that these sedimentary bands contain up to 50 per cent of iron sulphide, mostly in the form of pyrrhotite (Fe_7S_8). The geological structure is a dome, elongated in a NNW direction parallel to the regional strike, with the flanks dipping 45° to the east and west. Irregular stock-like bodies and flat-dipping dykes of porphyry are intrusive into all formations, including the nickel sulphide ore bodies. Most of the nickel sulphide mineralization occurs at the basal contact of the altered ultramafic with the underlying metabasalts, but a significant amount lies at higher stratigraphic levels within the ultramafic. The ore bodies are pyrrhotite–pentlandite associations with minor pyrite and chalcopyrite, and consist of a lower banded sulphide zone consisting entirely of sulphide minerals and an upper disseminated sulphide zone consisting of approximately equal amounts of sulphides and silicates and variable amounts of carbonate minerals[1].

Nickel deposits have been discovered at several other localities in the Norseman–Laverton greenstone belt (Fig. 2)—near Higginsville and Widgiemooltha south of Kambalda, at Scotia, Carr Boyd Rocks, Pyke Hill and Mount Clifford between Kalgoorlie and Laverton, and at Windarra and Mount Keith north of Laverton. All, with the exception of Pyke Hill (which seems to be the result of weathering of a nickeliferous serpentinite), are sulphide deposits, and all are associated with ultramafic intrusions and their metamorphosed derivatives, such as serpentinites and talc–tremolite schists. The most glamorized of these discoveries is the Windarra find, 15 miles north-west of Laverton on the property held by Poseidon N.L.

Nickel sulphide ores were discovered at Mount Windarra in September 1969 at the contact between an altered ultramafic (talc or talc–carbonate rock) and metasedimentary banded iron or jaspilite formation. This banded iron formation is exposed discontinuously along a low ridge for about 7 miles and there are occasional small outcrops of ultramafic rocks immediately adjacent to it on the east side. The contact strikes NNW but, because of the intense folding, is sinuous and dips steeply (80°–90°) to the east. Near the southern end of the outcrop area the dip flattens to 50° east. The Windarra ridge is on the east limb of a south-plunging anticline which, to the south, is completely covered by a thick alluvium layer. Part of the outcrop of the proved nickel sulphide body has been obscured by drift sand up to 30 feet deep which has accumulated against the Windarra ridge. The Windarra deposits, like the contact deposits at Kambalda, occur at the base of an ultramafic layer, but they differ from others in that banded iron formation (metajaspilite) rather than metabasalt forms the other wall. Nonetheless, it is interesting that banded ferruginous cherts, often with a high content of iron sulphide, occur in the associated country rocks at most deposits. This suggests that the sulphides of the chemically deposited sediments interbedded with the basaltic lavas were the source of the sulphur responsible for collecting nickel and copper from the later basic magma intrusions, as suggested by the sulphurization hypothesis of Naldrett[11], followed by the gravity separation of nickeliferous sulphides in the still-molten magma to form sulphide accumulations at the bottom of these intrusions.

It seems necessary, therefore, for the formation of these nickel ore deposits to have not only nickel-bearing ultrabasic intrusions, but sulphur-bearing country rocks such as pyritic or marcasitic sediments like the jaspilites, which are commonly associated with the submarine basalts of the early Archaean. But although some of the nickel ore bodies have been uncovered by mining operations, their mode of origin is still unknown.

Significance of Tertiary to Recent Land Surface

The southern half of Western Australia is an old, slightly dissected, lateritized land surface[12] dating from the middle or late Tertiary. The monotonous topography, characterized by long narrow salt lakes, elongated parallel to the regional structure, is relieved only by low ridges elongated in the same NNW direction, where resistant rocks such as jaspilites project above the general level. The lateritized surface is covered either by wind-transported sands, laterite, the completely weathered sub-laterite zone which extends to depths of the order of several hundred feet below the indurated laterite, or by Recent alluvial deposits resulting from sheet flood deposition in areas of arid erosion. It is this old land surface, with its highly weathered and lateritized cover, that has made mineral exploration in this country so difficult. The major gold mining centres of Western Australia are situated on ridges which lie near the laterite level, for only here could the prospector see the underlying rocks. The thick weathered cover and overlying alluvium and drift sand pose much the same problem to the present-day prospector, armed though he is with geochemical and geophysical techniques. As with the earlier gold discoveries in Western Australia, the most significant nickel finds, such as at Kambalda and Windarra, have been made in areas of relatively marked topographic relief, where the laterite cover is absent and the soils are residual in nature.

Lateritic nickel deposits occur at Wingelinna near the border of Western Australia with South Australia. Here the Giles Complex of mafic and ultramafic igneous rocks of Middle Proterozoic age has been lateritized, and secondary concentration of nickel has occurred along narrow zones representing infolded ultramafic layers along which the ultramafic rocks have been very deeply weathered. The Giles Complex rocks are deficient in sulphide minerals and the Wingelinna nickel deposits differ markedly from the sulphide mineralization of the Norseman–Laverton greenstone belt, with the possible exception of the Pyke Hill deposits, which have resulted from the weathering of nickeliferous serpentinites.

Because of the topography, the weathered nature of the country and the extensive alluvial cover, a small fraction only of the potentially nickeliferous areas (the "greenstone" belts) has yet been prospected for nickel.

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Meteorology and the Clean Air Act

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Since 1956 there has been a steady improvement in visibility in town and country in Britain, which can only be attributed to the decrease in the amount of smoke in the air. The Clean Air Act seems to have been the chief cause of this decrease.

THE problem of atmospheric pollution and its effect on the wellbeing of people living in towns and cities is not new; in Tudor times there was an outcry against burning coal in London, and in December 1671 the diarist John Evelyn[1] wrote of the "thickest and darkest fog ever known". During a foggy week in December 1873 the death rate in the Administrative County of London was 40 per cent above average, and there were similar increases during fogs in 1880 and 1892. The continued growth and spread of London during the first half of the twentieth century intensified and extended the menace of pollution; for example, the great fog of December 1873 hardly affected Croydon, but the fog in December 1952 resulted in a sharp increase in mortality in that town. This fog of December 1952 was estimated to have been responsible for 4,000 deaths in the County of London; during the week of fog, deaths from bronchitis increased about nine times and from pneumonia nearly four times. These were much greater increases than previously recorded in London fogs. As a consequence there was intensification of the campaign for smoke abatement and the Committee on Air Pollution was set up in July 1953. The eventual result was the passing of the Clean Air Act 1956, which gave local authorities powers to establish smoke control areas.

Fogs are natural phenomena and apart from reducing visibility they are fairly innocuous, although they can be troublesome to people with afflictions such as chronic chest complaints. Medical experience, however, leaves no doubt that dangerous and harmful conditions occur when pollution is present in natural fog in sufficient quantity to produce "smog". Smoke and other forms of pollution thicken and contaminate fog, and increase its persistence and frequency in several ways. They provide nuclei for the formation of liquid fog particles and, even if the air is not moist enough for condensation, the smoke alone may be sufficiently dense to reduce visibility below the fog limit, provided the vertical stability is great enough and the winds light enough to prevent dispersal. The smog cuts off sunshine, thereby retarding the natural processes of heating and evaporation which would disperse the fog. The weather factors most likely to be improved by application of the Clean Air Act were therefore visibility and, to a lesser extent, sunshine. This seems to have been borne out by studies of the temporal variation of visibility and fog frequency in many "black areas" and by some work on London sunshine.

Fog in Greater London

In 1964 I[2] compared the frequency of dense fog (visibility less than 55 yards) and thick fog (visibility less than 220 yards) in central London (Kingsway) and outer London (Heathrow and Kew) during 1947–1962 (Table 1).

Chandler's map[3] of the "percentage fog frequency in London 0900 GMT September 1936–March 1937" showed that the frequency was least in the suburbs, and increased towards the centre of the city. Table 1 indicates that this situation had been reversed even before the Clean Air Act of 1956. The low frequency of fog in the city centre compared with the outskirts is attributed chiefly to the greater urban heat effect in the centre, but the reduction of pollution in central London probably also played a part. Before and, to a greater extent, after the Second World War, development in central London resulted in the replacement of many old coal-fired houses by large blocks of offices or flats heated principally by smoke-free methods. These developments increased the urban heat effect, and the combination of increased environmental heat and decreased smoke led to a reduction of fog. As Table 1 shows, a comparison of figures for 1947–1954 with those for 1955–1962 shows that the decrease in the frequency of dense fog was substantially greater at Kingsway than at Heathrow or Kew. To a lesser extent this also applies to thick fog.

Investigation showed that the mean winter urban heat effect at Kingsway was about the same during the two eight year periods, and that 77 per cent of the observations of dense fog there during the whole sixteen years were associated with winds of only one knot or less, suggesting that on occasions of dense fog local pollution was much more important than pollution drifting from elsewhere. It is very unlikely that the difference between inner and outer London was due to any synoptic variation. Local authority records show that, in general, smoke control commenced earlier and progressed faster in the area around Kingsway than in the areas around Kew and Heathrow, and it is reasonable to assume that this was the principal cause of the marked decrease in the frequency of dense fog at Kingsway during 1955–1962.

Wiggett[4] found that the mean frequency of observations of visibility 440–1,090 yards at London (Heathrow) Airport decreased by nearly 40 per cent during 1946–1963, and that there was a smaller decrease in the middle visibility ranges—about 10 per cent for visibilities between 45 and 105 yards. But there was no significant trend for visibilities less than 45 yards; this agrees with my figures for dense fog at Heathrow (Table 1).

Wiggett suggested that the fogs causing visibility of 440 to 1,090 yards were chiefly smoke fogs, and that the decrease in frequency was due to a reduction in smoke pollution, while the fogs with smaller visibilities were water fogs. The latter, however, must have been contaminated

Table 1. AVERAGE ANNUAL NUMBER OF HOURS OF DENSE AND THICK FOG AT KINGSWAY, HEATHROW AND KEW DURING 1947–1954 AND 1955–1962

		1947–1954	1955–1962	Percentage decrease
Dense	Kingsway	27	13	52
	Heathrow	57	54	5
	Kew	95	73	23
Thick	Kingsway	68	52	24
	Heathrow	202	177	12
	Kew	241	189	22

Table 2. AVERAGE ANNUAL NUMBER OF HOURS OF DENSE AND THICK FOG AT KINGSWAY AND HEATHROW DURING 1947–54, 1955–62 AND 1963–69

		1947–1954 (8 years)	1955–1962 (8 years)	1963–1969 (7 years)	Per cent decrease 55–62 to 63–69
Dense	Kingsway	27	13	2	85
	Heathrow	57	54	18	67
Thick	Kingsway	68	52	15	71
	Heathrow	202	177	83	53

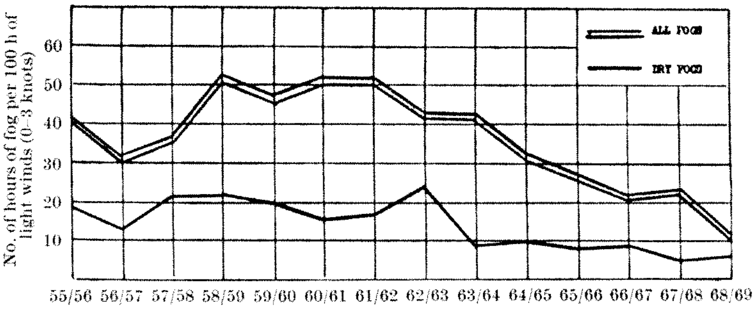

Fig. 1. Number of hours of fog (visibility < 1,000 m) and "dry" fog (visibility < 1,000 m and relative humidity < 95 per cent) per 100 h of light winds (0–3 knots) at Glasgow Airport in each winter from October 1955. (From *J. Nat. Soc. Clean Air*, **151**, 26; 1969.)

by smoke, and it may be that, although the reduction in pollution was sufficient to affect slight smoke fogs, it was not yet great enough to influence the frequency of dense fogs or smogs. Later observations tended to support this idea. Freeman[5], studying the frequency of thick fog, fog and mist at Heathrow during 1949–1967, confirmed the decrease in the frequency of fog[4] and also found that there had been a notable decrease in the frequency of visibilities less than 99 metres (about 108 yards) since about 1963. He concluded that the effects of the Clean Air Act (1956) in reducing smoke in the London area were chiefly responsible for the progressive decrease of fog at Heathrow.

If the figures for thick and dense fog at Kingsway and Heathrow are brought up to date (Table 2) it is clear that the marked improvement at Kingsway between 1947–1954 and 1955–1962 has continued and intensified, and that the same applies to Heathrow during 1963–1969. There seems little doubt that this is associated with the continued spread of smoke control measures under the Clean Air Act.

Manchester, Yorkshire and Glasgow

Atkins[6] has compared the frequencies of visibilities less than 220, 440, 1,100 and 2,200 yards at Manchester (Ringway) Airport during October to March in 1949–1954, 1955–1959 and 1960–1964, allowing for variations in the frequency of light winds (0, 1–3, 4–6 and 7–10 knots) from one period to the other. The frequency of each of the four classes of visibility during 1960–1964 was less than the corresponding frequencies during the two other periods. There seems to have been a noteworthy change about 1960, with light winds becoming less likely to be accompanied by poor visibility than before. It is reasonable to attribute this change to the operation of the Clean Air Act in north-west England. In 1960[7] the Ministry of Housing and Local Government estimated that about half the premises in Manchester would be in smoke control areas by the end of 1963.

Corfield and Newton[8] made a statistical analysis of visibilities in relation to wind direction at Finningley, an aerodrome to the east of the industrial part of the West Riding of Yorkshire, omitting occasions of precipitation. The wind directions, omitting calms, were grouped into 30 degree sectors to include sources of smoke pollution within 40 miles of Finningley. The chief sources of pollution are sector 235°–265° (Sheffield, Rotherham); sector 265°–295° (Doncaster, Barnsley, Mexborough, Huddersfield and Halifax); sector 295°–325° (Doncaster, Wakefield, Pontefract, Castleford, Dewsbury, Halifax, Bradford and Leeds).

Between 1958–1961 and 1962–1965 there was a marked decrease in the frequency of poor visibility (≤ 3,000 yards) and a marked increase in the frequency of good visibility (> 10 nautical miles) with winds in sectors 235°–265° and 265°–295°. But when winds were in the sector 295°–325°, although there was a decrease in the frequency of visibility ≤ 3,000 yards, there was a sharp increase in the frequency of visibility between 3,000 yards and 2½ nautical miles with little change in the frequency of other ranges. In connexion with these results, which can be linked with smoke control, it is interesting that Sheffield (235°–265°) is closer to Finningley than Leeds (295°–325°) and that, by 1965, almost 60 per cent of the buildings in Sheffield were under smoke control regulations compared with less than 20 per cent in Leeds, although the figure was about 40 per cent for Bradford which is still further away from Finningley. Although there are difficulties in interpreting the results, there is strong evidence that the improvement in visibility at Finningley is chiefly a consequence of smoke control.

In 1969, McKellar[9] examined the frequency of fog at Glasgow Airport during the winter (October–March) from 1955–1956 to 1968–1969, accepting the definition of fog as visibility less than 1,100 yards, and assuming "dry fog" to be fog with relative humidity less than 95 per cent. To allow for the variation in the frequency of light winds, results were expressed as the number of hours of fog per 100 hours of light winds (0–3 knots, Fig. 1). According to McKellar smoke control in some parts of the Glasgow area commenced in 1959 and the smoke control areas have been extending ever since. Fig. 1 shows a continuous reduction in the frequency of fog since 1961–1962 (1962–1963 for dry fog); smoothing the curves by taking pairs of winters, the average fog frequency for 1967–1968 and 1968–1969 was only about 17 hours per 100 hours of light winds compared with about 50 for 1959–1960 and 1960–1961. This is a reduction of about 66 per cent; the reduction for "dry fog" is about the same. Looking at occasions when the wind at the airport blew from the main areas of pollution (020°–100°) at speeds of 7–10 knots, and comparing visibilities during the winter of 1968–1969 with average conditions during the winters of 1958–1959 to 1961–1962 (Table 3), McKellar concluded that the visibility in Glasgow had improved significantly, presumably because of the operation of the Clean Air Act.

Fog in the United Kingdom

When Dinsdale[10] compared the frequency of fog at several rural and urban sites during 1947–1967 he demonstrated a general decline in fog frequencies in each case. He warned that changes in fog frequencies anywhere should be viewed against this background, and that the possibility of synoptic variations should not be overlooked. Fig. 2 shows the variation of the frequency of fog (visibility < 400 yards) at ten places in the United Kingdom during 1958–1967. The decrease in frequency at Elmdon (Birmingham Airport) is particularly outstanding. It is doubtful whether Dinsdale's assumption that stations such as Abingdon are "little affected by smoke" is correct. The aerodrome weather gazetteer for Abingdon states "the various smoke sources which have

Table 3. PERCENTAGE FREQUENCY OF VISIBILITY RANGES WITH WINDS OF 7–10 KNOTS FROM 020°–100° AT GLASGOW DURING THE WINTER (OCTOBER–MARCH)

Average	< 1,000 yds	1,000–1,999 yds	2,000–3,999 yds	4,000 yds–4 mls	4–8 mls	8–12 mls	12–20 mls	> 20 mls
Oct. 1958 to March 1962	4	35	40	21	0	0	0	0
Oct. 1968 to March 1969	0	3	43	26	20	8	0	0

noticeable effect on visibility at Abingdon lie in a wide sector which extends from north-west, through north and east to south" and "when light winds from NNW are maintained for a day or longer during the winter, smoke haze from the industrial areas around and beyond Birmingham becomes thick and persistent with little diurnal variation in visibility". It must be remembered that social changes such as blocks of flats instead of rows of houses, the spread of central heating and the increase in the number of working wives with a consequent decrease in all-day fires have all contributed to a diminution of smoke in many other towns and cities as well as in the "black areas". I do not suggest that drifting smoke would be thick enough to reduce visibility below 400 yards at all rural sites such as Abingdon, but it would tend to increase the persistence of natural fogs and any diminution of this smoke would therefore result in less frequent fog. Thus a diminution of smoke from "black or other areas" would result in a decrease in the frequency of fogs at both urban and rural sites. Although Dinsdale's caution is timely, I feel that his results support the thesis that the recent improvements in visibility are, to a large extent, due to smoke abatement.

Sunshine in Greater London

In 1969 Jenkins[11] noted the increase of sunshine in London during 1958–1967. He compared the increase in central London, as represented by Kingsway (this station was moved a quarter of a mile to Holborn in January 1965), with the recorded sunshine in the suburbs, as represented by Kew, and at a rural site near London, as represented by the Royal Horticultural Society's gardens at Wisley. Kew is about 9 miles WSW and Wisley about 21 miles SW of central London. The average monthly sunshine during the ten years 1958–1967 was expressed as a percentage of the thirty year average 1931–1960 (Fig. 3). The difference in amounts of sunshine at the three places was relatively small during the summer half year April to September, when domestic air pollution is always at a minimum. During the winter half year October to March, however, there was a marked increase in average sunshine at Kingsway, a much smaller increase at Kew and hardly any increase at all at Wisley. This fits in with the faster progress in smoke control in central London than in the suburbs. The average sunshine at Kingsway since 1958 during November to January has increased by about fifty per cent above the long term average (1931–1960) and average annual sunshine is now about the same at Kingsway, Kew and Wisley. This improvement is most likely associated with the decrease in smoke resulting from the Clean Air Act.

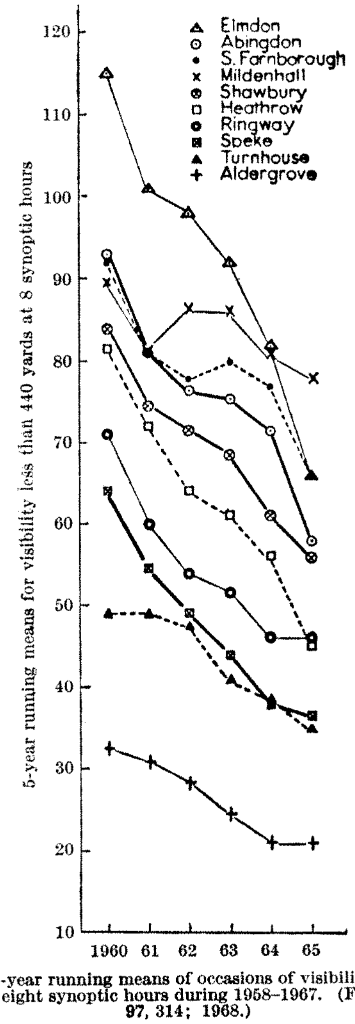

Fig. 2. Five-year running means of occasions of visibility less than 440 yards at the eight synoptic hours during 1958–1967. (From *Met. Mag.*, **97**, 314; 1968.)

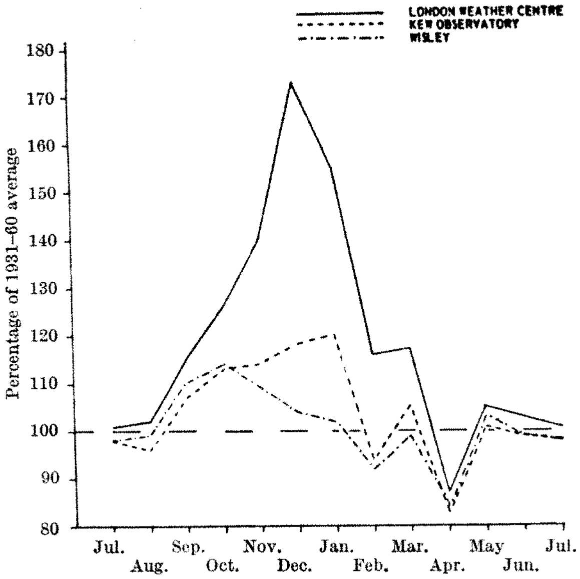

Fig. 3. Average monthly sunshine at the London Weather Centre, Kew Observatory and Wisley during 1958–1967 as a percentage of the average for 1931–1960. (From *Weather*, **24**, 2; 1969.)

Clearly there has been, during the last decade or so, a steady improvement in visibility at both urban and rural sites, particularly marked in some "black areas". In such areas the reduction in the frequency and intensity of fog has been dramatic. There has also been an outstanding increase in winter sunshine in central London during the same period. There seems little doubt that these improvements in weather are associated with smoke abatement, both voluntary and enforced, and it seems fairly certain that the Clean Air Act of 1956 is chiefly responsible for the dramatic improvement in certain towns and cities; the term "black area" no longer applies to these places.

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Genes and Hereditary Characteristics

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This appraisal of ideas about genetic determination is based on Dr Hershey's report in the sixty-eighth *Year Book* of the Carnegie Institution of Washington, issued last February.

INFLUENTIAL ideas are always simple. Since natural phenomena need not be simple, we master them, if at all, by formulating simple ideas and exploring their limitations. The notion that genes determine the characteristics of biological individuals and species is exceptional among simple ideas: its limitations have consistently diminished with the passage of time. In this article I shall consider both the simplicity and the limitations of ideas about genetic determination.

The early students of heredity were forced to distinguish between the genetic constitution of an animal or plant (its genotype) and the expression of its genes in visible characters (its phenotype)[1]. The distinction is particularly clear in an individual that received dissimilar genes from its two parents: the dual genotype clearly gives rise to a single phenotype. But even an individual with a single set of genes has a phenotype that depends on its stage of development, environmental influences and accidental factors.

The notion that genotype controls phenotype is pure tautology in the typical breeding experiment in which genotype means differences between genotypes, and phenotype means differences between phenotypes. Mendel brought the tautology to light by showing that inheritance depends on unitary factors. Morgan and his students traced these factors to hypothetical genes at known locations in visible chromosomes and created the science of formal genetics[2]. The generality of Mendel's discovery can be summarized by saying that we know of no biological characters, including developmental patterns, that are immune to gene mutations. The importance of his discovery lay in showing that inheritance could be analysed.

Of necessity, formal genetics largely ignored questions about gene function. "As to the nature of 'genes' it is as yet of no value to propose any hypothesis; but that the notion 'gene' covers a reality is evident from Mendelism." This statement, quoted from Johannsen[1], sufficed pretty well for three decades. E. B. Wilson in his famous book expressed a more reckless opinion[3]. "No one . . . now conceives the chromosomes as exclusive agents of heredity. They may be considered as modifiers in a reaction system. . . . Chromosomes are to be regarded as *differential factors of heredity*, rather than central governing elements. . . ." The relation between genotype and phenotype for many years seemed infinitely complicated, as indeed it was in the prechemical era of genetics.

Help from *Neurospora*

In 1940 Beadle and Tatum turned new light on Mendel's unit factors when they described their first experiments with the bread mould *Neurospora*. This organism was unique at the time in permitting both genetic and nutritional experiments. With it Srb and Horowitz[4], for example, could show by mutational analysis that each biochemical step in the synthesis of the amino-acid arginine is the province of a single gene functioning in the production of a single enzyme. Results of this sort, epitomized in the phrase "one gene–one enzyme", obviously conflicted with what everybody knew: that the relation between genotype and phenotype was infinitely complicated. Chiefly because of this conflict, and partly too because the meaning of the phrase was not precisely defined, the new hypothesis met with strenuous opposition.

Actually, the hypothesis consisted of two parts: only one gene functions specifically in the synthesis of a single enzyme; and one gene functions specifically in the synthesis of only one enzyme (if any). Both parts were necessarily a little vague because the significance of the word "specifically" was not clarified for another decade or more. (We now say that one gene determines the amino-acid sequence of one enzyme.)

I do not suppose it is possible to assign a date to the eventual acceptance of the one gene–one enzyme hypothesis, but I remember clearly the Cold Spring Harbor Symposium of 1951 at which the controversy reached its climax. At that meeting Horowitz and Leupold[5] presented their paper entitled "Some Recent Studies bearing on the One Gene–One Enzyme Hypothesis", which would be better known today if it had been called "Confirmation of the One Gene–One Enzyme Hypothesis by the Use of Conditional Lethal Mutations". In the stormy discussion that followed I was unable to grasp the issues, but it was clear to me that nearly everyone was quarrelling with Beadle and Tatum, who were absent, and ignoring the results just presented.

Horowitz and Leupold offered rather subtle arguments in support of the hypothesis that only one gene functions "in a direct manner" in the synthesis of a single enzyme. They also showed that there are relatively few genes concerned with functions common to synthesis of proteins in general, and proposed that the immediate precursors of proteins are single amino-acids or their derivatives, not polypeptides. Their paper is historic both for cogency of argument and because it describes the first systematic use of temperature-sensitive mutants.

Chemical Genetics

In the context of the one gene–one enzyme hypothesis, subsequent developments were chiefly the discovery of two smaller classes of genes: regulator genes[6], the products of which in the well known examples are proteins interacting directly with DNA to interfere with the expression of other genes[7,8]; and genes in which the structures of ribosomal and transfer RNAs are encoded[9]. At present there are no clear indications that more classes remain to be discovered, although, of course, not all proteins determined by genes are properly called enzymes.

Genetics was a perfected theoretical discipline some time before chemical genetics was invented, and no real conflict between them was possible. (Read, for example, Wright's review[10], then Beadle's[11].) The initial aim of chemical genetics called for elucidation of the functional basis of the determination of phenotype by genotype. This aim has been achieved in large measure, largely through elucidation of the structure of DNA[12]. The chief element of surprise, I think, was the simplicity of the denouement, anticipated in part by Beadle and Tatum.

If the overall plan is simple, it ought to be possible to put it into a few words, which I attempt as follows. First, the genotype resides in DNA—more importantly, in the linear sequence of the four nucleotides in single DNA strands. Second, nucleotide sequences in single DNA strands are transcribable into complementary sequences according to simple one-to-one rules: the four bases form only two interstrand pairs, guanine–cytosine and adenine–thymine. This code or its equivalent is used for DNA replication, gene transcription, and synthesis of ribosomal and transfer RNAs. It also regulates the structure of typical double stranded DNA molecules.

Third, sequences in one of the two complementary strands, transcribed into messenger RNA, are translatable into amino-acid sequences in proteins according to a second code unrelated to the first. This is a non-overlapping triplet code (three bases per amino-acid) usually called the genetic code.

Fourth, the phenotype, to the extent that it is understood at all, depends on the structures of species-specific proteins, structures that depend in turn on linear sequences of amino-acids. This conclusion is a necessary inference from what is known of enzyme control of metabolism, on the one hand, and the nature of the genetic code, on the other. That amino-acid sequences determine protein structure might have been guessed years ago from the fact that thermal inactivation of enzymes is often reversible. It is now being proved by artificial synthesis of enzymes. Moreover, gross biological structure can be directly determined by subunit structure, as seen in the reconstitution of virus particles from their parts[13,14]. These successes demonstrate that three dimensional structures can be represented in one dimensional messages, and thus verify one of the most important implications of the Watson–Crick model of DNA—an implication that familiarity with human speech had scarcely prepared us for.

My summary is necessarily abstract. Perhaps I should mention one concrete fact. Rüst and Sinsheimer[15] have shown that either complementary strand of DNA from the phage φX can infect bacterial cells to give rise to identical viral progeny. Therefore the two strands contain the same genetic information: they encode the same genotype. If these two strands could be examined in detail, they would be seen to differ from each other in a systematic way: each adenine residue in one is matched by a thymine residue at the corresponding position in the other, and similarly for the pair guanine and cytosine. This is certain, even though nucleotide sequences cannot be read directly. How we know is fairly simple, too, but the evidence cannot be put into a few words.

What Critics Say

So far I have made what I consider to be factual statements. Perhaps the best way to assess my judgment in this matter is to look at the criticisms raised by people who see things differently. Carl Lindegren, a perceptive man with a brave disregard for the rules of debate, once pointed out that the city of Chicago existed for some time before it became dependent on electricity[16]. He inferred that if DNA happened to be a late-comer on the biological scene, contemporary research would be hard put to discover that fact. Lindegren's analogy is not very apt, for towns do not acquire public utilities by inheritance. Nevertheless, it is fair to ask why biologists should believe in something not visible in the historical record. The answer, to which molecular genetics has contributed something, can be given as follows.

If we assume kinship of living things, we at once imagine an evolutionary family tree stemming from an aboriginal branch point that represents an event of singular importance. That event, the invention of hereditary differences between lines destined to persist, I take to be conceptually equivalent to the origin of life. If we find DNA in both aboriginal branches, we conclude either that DNA function antedates the origin of life or that DNA was independently created two or more times. In fact, biochemists find a common genetic code exploited in all members of a reasonable sample of biological species. To most people, the hypothesis of unique origin provides the only economical explanation. (My weak statement reflects a certain lack of rigour in the argument. Strictly speaking, every homological explanation has an analogical equivalent.)

The same issues are raised in extreme form when experimental evolutionists suggest that life is being continually recreated. There are really two issues. On the one hand, the unity of biology speaks for unique origin. On the other hand, the complexity of the simplest forms of life suggests an inordinately low frequency of spontaneous generation: only once on Earth as far as we know and perhaps not at all in an unexplored universe. I am aware that these arguments are logically ambiguous. I am also aware that they were advanced before the phrase "molecular genetics" was coined. But I wish to make two less obvious points. First, in spite of the simplicity of the overall plan of inheritance, recent advances in molecular genetics serve only to augment, not diminish, our appreciation of biological complexity. For example, Peter Lengyel recently added up 130 known macromolecular components necessary just for the synthesis of protein. Second, without evidence for the unity of biochemistry, now clearly evident in the universality of the genetic code, the doctrine of the unity of biology would be insecure indeed, particularly with respect to the simpler forms of life. In short, life exists and we infer that it had a beginning. To find anything credible in either the fact or the inference is to miss the point.

Barry Commoner has devoted more thought to the search for weaknesses in molecular biology than anyone else (except, of course, molecular biologists). Recently[17] he cited the discovery by Speyer that mutations affecting Kornberg's DNA polymerase (an enzyme concerned with DNA synthesis) can influence the rate of further mutation. This fact shows, according to Commoner, that heritable characteristics are determined in part by nucleotide sequences in DNA, in part by the enzymes that reproduce those sequences. He has also remarked somewhere, presumably to defend his inference against obvious forms of attack, that it has not been shown that information theory is applicable to biological problems. Both these statements strike me as irresponsible: they are not untrue, just obtuse and misleading. Because Commoner is an eloquent writer[18], and has used such phrases as "theoretical crisis", "illusory successes of molecular biology", and "collision course" to manufacture conflict between "two kinds of biology", it seems worthwhile to try to straighten out the technical basis of his argument. Fleischman[19] has made one attempt. My own is a little simpler.

Long before 1953 it was evident that general mutation rates are themselves determined by genes. Geneticists described the situation as follows. The characteristics of organisms are determined by genes received from their parents and transmitted to their progeny, genes that must, therefore, be duplicated in each cell generation. But genes are not perfectly stable, or are not duplicated with perfect fidelity, whence arise the mutations that serve evolutionary purposes. Thus it is necessary to distinguish between a mechanism of duplication on which inheritance depends, and occasional mistakes made by that mechanism, few of which can be put to use. The frequency of error depends on all sorts of things—external radiation, exposure to various chemicals, temperature, ionic milieu, as well as genetic constitution itself—including, we now know, the gene-determined structure of at least one enzyme. Thus information theory, insofar as it is needed in this connexion, is just the common sense of geneticists, who saw fit to distinguish between speech and noise. I am forced to conclude that Commoner is quarrelling with the principles of genetics, and that he has not said anything about molecular biology.

Reply to the Critics

To separate my counter-criticism from the arbitrary meanings of words, I repeat it in a form that avoids loaded phrases. I have noted that agencies external to the cell, such as sources of radiation, affect mutation rates. According to Commoner's reasoning we ought, on that account, to abandon the notion that living cells can reproduce themselves.

There are, I think, more interesting limitations to current biological principles than anything pointed out by the carpers. The discovery by early geneticists that a unique set of genes determines the characteristics of the individual at once raised the question whether or not biological traits are determined exclusively by those genes. This question has never been answered and is difficult to phrase intelligibly. The demonstration by molecular biologists that a linear genetic message can be translated into three dimensional structure, as in the multiplication of virus particles, showed that in principle the known mechanisms of inheritance could be the only mechanisms. (The example of the viruses is important because there one can observe the regeneration of quite different viral species in the same cellular milieu, depending only on the kind of DNA molecule introduced at the start.) The inference that all three dimensional structure is encoded in nucleotide sequences does not necessarily follow, however. I shall call that inference the unwritten dogma, for it must be shared at least by those biologists who consider molecular biology all but finished. I turn now to some problems of cellular organization that seem to lie beyond the reach of current molecular principles. Here, if anywhere, one might expect to enter the province of biological theory.

The cell theory, dating from 1839 or earlier, engenders lengthy discussions in textbooks, discussions that are interesting but do not, I think, succeed very well in stating a theory. The central notions are: that living things come in cellular form, that cells arise only from pre-existing cells, and that all cells are homologous. Without the last proviso, the cell theory is only a historical remnant of controversy about facts. The last proviso itself comes from Darwin, not cell theorists. Discussion of the theory commonly ends with the statement that the cell is "the structural unit of life", or even "life's minimum unit"[20]. Such statements serve chiefly as a reminder that scientific theories cannot be profitably discussed outside their proper experimental context.

According to Tartar[21], the pertinent experiments are nearly as venerable as the cell theory. For example, Gruber (1885), pursuing earlier work, showed that a single cell of the protozoan genus *Stentor* could be cut into three parts, from each of which a complete animal would regenerate. In subsequent experiments with other ciliates, pieces as small as 1/80 of the cell volume were found capable of regeneration. Such experiments show that the cell is a homoeostatic unit of life, not a minimum unit, and raise the question, what is the minimum unit ? By suppressing this question, the cell theory in effect keeps living things out of the laboratory.

More useful theories divide the cell into conceptually distinct parts in the attempt to identify partial functions. Here the successes, notably enzymology and genetics, should not be allowed to overshadow the failures, such as analysis of cellular development, which also has a long history. Tartar ascribes to Prowazek (1904, 1913) what I take to be the traditional view: "Guidance of the elaboration of formed parts in the cytoplasm is to be sought in neither a nucleus of unprescribed location nor a flowing endoplasm but in the most solid portion of the cell, namely, the ectoplasm"[21]. This simplifying hypothesis divides the cell into two parts: on the one side, genes and gene products; on the other side, a cell cortex or skeleton that seems to manifest supramolecular properties. Strictly speaking, the hypothesis says nothing about the origin of the guidance system, which could in principle be recreated every time an egg is fertilized, and whenever a protozoon emerges from its cyst. But some sort of primary guiding principle is usually assumed. For example, Luria[22] remarked, in effect, that just as biological evolution is separate from cultural evolution, so is DNA evolution separate from the evolution of cellular organization. This I suspect is as close as it is possible to come to a contemporary statement of the cell theory. Like all good theories, it admits alternatives: for example, the unwritten dogma, according to which biological evolution is solely the evolution of nucleotide sequences. These alternatives were presented in essentially the same terms by Sonneborn[23].

The hypothesis of bipartite inheritance arose naturally, I think, from the fact that cellular differentiation can be reversible or not to any degree. The reversible changes seemed to imply extragenic determination and the irreversible changes suggested extragenic inheritance, that is, differentiations dating from the origins of the species. The force of this argument is now weakened by the evidence that developmental changes sometimes can be explained in terms of selective control of gene action[24]. But not all persistent changes in cell structure and behaviour can be so explained[24,25]. Before turning to the experiments, I call attention to the purely logical difficulty of imagining an experimental result requiring the hypothesis of bipartite cellular inheritance.

Acquired Traits Retained

There can be no question about evidence that certain acquired traits can persist in a cell lineage. This is not new but improved evidence, which should not be allowed to confuse us. To say that some acquired traits are inherited may be literal truth, but it sacrifices the purity of language that comes to us from Mendel: all traits are acquired, no trait is inherited, not even DNA structure is inherited, only hereditary principles are inherited, for example, nucleotide sequences. All this was explained by Johannsen[1], questioned by Wilson[3], and justified when it was found how genes really work. Only then could one invoke information theory to distinguish between what is inherited and what is merely indispensable. The possibility that cells utilize extragenic sources of information should, of course, be kept open, but it must be admitted that no one has found two biological species with identical genomes, nor has anybody proposed a plausible model for extragenic inheritance. (The notion of the primer[26], that is, a structure that is both substrate and product of enzyme action, is promising but rudimentary.)

If cells draw on an extragenic source of information, a second abstraction must be invoked, another vital principle superimposed on the genotype. A likely candidate already exists in what is usually called cell polarity, which tradition places in a rigid ectoplasm for good reason —it is a spatial principle, and as such requires mystical language. Seemingly independent of the visible structures that respond to it, polarity pervades the cell much as a magnetic field pervades space without help from the iron filings that bring it to light. Biological fields are species specific, as seen in the various patterns and symmetries of growing things[27]. The existence of polar fields is best seen in experiments with *Stentor*, whose ectoplasm contains figurative iron filings in the form of pigmented granules. The following description is condensed from Tartar's book[21].

Stentor coeruleus is an aquatic one-celled animal bearing feeding organs at its head end and a hold-fast at its tail end. The entire surface is marked by longitudinal stripes of two kinds: clear stripes carrying cilia, alternating with granular stripes without cilia. During the life of the animal, its cortical stripes grow both in width and in number. The splitting of old (wide) stripes into young (narrow) ones occurs on the ventral surface and proceeds asymmetrically, producing a circumferential (left–right) gradient of stripe widths meeting as a visible boundary on the median ventral surface.

Before each cell division, and during regeneration of decapitated animals, new mouth parts start to form in the region of stripe multiplication on the ventral surface. (The parts afterwards migrate to their proper position.) These observations define an oral primordium site lying near the junction between wide and narrow stripes. However, this site does not contain an indispensable structure, because the dorsal half of a longitudinally bisected animal regenerates a new primordium that appears in the newly created junction between wide and narrow stripes. Apparently the oral primordium develops when required at the poles of a left–right gradient, and near the equator of a longitudinal gradient.

Likewise the hold-fast comes not from a specific hereditary primordium but from something generated at the posterior pole of a longitudinal gradient. Thus if the hold-fast is tucked forward by surgical means, a second one develops at the newly created posterior pole. Animals with two mouths and one tail, or with two tails and one mouth, once created by surgical interference, reproduce as biotypes with varying degrees of stability.

A piece of cortex grafted in reverse orientation into an animal may rotate to restore normal polarities, may degenerate and disappear, or may develop its own mosaic stripe pattern. Similarly, an animal bisected transversely, and reconstructed with the head portion rotated 180° with respect to the tail, may regain its normal stripe pattern either by rotatory slippage of the two halves with respect to each other or by replacement of stripes in one of the halves through outgrowth from the other.

These experiments, like comparable experiments with *Paramecium*[24], show that polarity resides in all parts of the cell cortex. Perhaps, as many people think, polarity represents something that was invented only once and has evolved since on its own.

The well known experiments of Hämmerling on *Acetabularia*[27] set limits to the hypothesis of bipartite inheritance. In this unicellular alga the single nucleus lies at the base of an erect stalk, well separated from the growing tip. At the tip of the stalk a cap eventually forms as part of an elaborate reproductive process. The structure of the cap is species specific, and interspecies grafts show that cap structure is determined by materials originating in the nucleus or rhizoids and migrating up the stalk, to the exclusion of outgrowths from the tip. Cortical inheritance, if it exists in *Acetabularia*, is apparently not species specific. Perhaps, contrary to what many people think, polarity resides exclusively in the gene-determined structures of polar molecules, and only genes evolve. If so, the only cell theory worthy of the name is wrong.

In *Stentor* and certain other cells, all reasonably large pieces of the cell cortex are equipotent with respect to regeneration of cortical patterns—patterns that are, moreover, subject to metastable variations. In bacteriophages, supramolecular patterns do not persist as such but recur, apparently residing exclusively in the gene-determined structures of individual molecules. *Acetabularia* presents a tantalizing example somewhere in between. Taken together, the facts encourage us to see in cortical polarity a historical invention that ought to be analysable in terms of structure and process. They do not encourage us to think that the task of molecular biology is finished, even at the cellular level. In Tartar's words[21], "Our greatest lack and most fruitful opportunity in biology lies in conceiving and testing the nature and capabilities of persistent supramolecular patterns".

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Marijuana Intoxication: Common Experiences

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As a guide to future experiments, the chief experiential effects of marijuana have been elucidated with the help of a detailed questionnaire given to seasoned marijuana users whose experiences, it seems, are almost entirely pleasant.

THE widespread use of marijuana, chiefly among college students, has raised important ethical, social and scientific questions and has stimulated some research. There is a risk, however, that studies will be unrepresentative of the effects of marijuana as it is actually used. One pitfall is the attempt to study "pure" effects in which extraneous sources of variation are minimized. Thus the report on one otherwise excellent laboratory study[1] says the "... greatest effort was made to create a neutral setting . . . verbal interactions between staff and subjects were minimum and formal . . .".

An adequate understanding of marijuana intoxication which focuses on particular effects experienced by a particular person must take into account (1) long term characteristics of the user—his personal and physiological idiosyncrasies; (2) immediate expectations and desires about what will happen during intoxication; (3) past experiences with marijuana and other psychoactive drugs as well as learned skills for modifying the drug experience; (4) immediate emotional state; (5) the social and physical setting; (6) the amount of marijuana used; and (7) chemical variations of the marijuana used. (Many of the marijuana users I interviewed insisted that certain samples of marijuana were qualitatively different from one another, and brought about different effects—laughter or sedation, for example.) During intoxication, these variables may alter as, for example, a user's companions direct his attention to new effects. Knowledge of how these variables affect marijuana intoxication is meagre, and is based almost exclusively on anecdotal accounts[2-5].

Some of the effects of marijuana may be evident only when the variables mentioned above have certain values. Other effects, which may be called "pure drug effects", may be caused exclusively by the pharmacological action of the drug. Both types are equally "real" and interesting. The traditional "neutral" setting of the laboratory, however, can provide a very limited configuration of determining variables: thus many potential effects will not show up in the laboratory situation, so that the picture of marijuana intoxication obtained there may be only partial. Indeed, it has been argued that the laboratory setting positively inhibits many important human manifestations[6-9].

These disadvantages can be overcome, however, if the investigator knows the entire normal range of effects of marijuana. My purpose here is to record this range for the benefit of future investigations. I designed a questionnaire on the basis of what I had read and learned from informal interviews with marijuana users. The questionnaire was answered by experienced marijuana users with respect to all their experiences over the preceding six months. The variables I have referred to would have been operative throughout their ranges, giving an adequate sample of the potential effects as well as the pure drug effects.

Informal interviews, chiefly with students, were carried out for two years to find out what marijuana intoxication was like. The results were combined with other descriptions of effects (see ref. 10) to give 206 descriptions of possible effects. The descriptions made up the chief part of a large questionnaire. The respondent was asked to say how often he had experienced each possible effect during the preceding six months. (The restriction to the last six months of use was intended to reduce lapses of memory. Respondents were also asked to estimate the smallest degree of intoxication necessary to experience each effect on a five-point scale: these data and more detailed analyses will be presented elsewhere.) If the description made no sense to him, he could skip it; otherwise he was asked to estimate frequency in one of five categories: (1) never; (2) rarely; (3) sometimes (between 10 and 40 per cent of time); (4) very often (more than 40 per cent of time); and (5) usually (almost always). The rest of the questionnaire consisted of questions on background (age, sex, education and experience with other psychoactive drugs).

The questionnaires were distributed by students, who were asked to pass the questionnaires on until they reached experienced marijuana users; this method was intended to ensure anonymity. A stamped return envelope was attached to each questionnaire and, of about 750 questionnaires, 153 were returned.

A letter that went out with the questionnaire was written colloquially and used the drug culture terminology. It explained the purpose of the study, asked the respondents to be as accurate as possible, and offered to make results available to anyone who wanted them after a year. It asked that only people who had used marijuana at least a dozen times should fill in the questionnaire, to avoid problems of adaptation.

Methodological Doubts

Are the answers valid ? For example, can the respondents adequately assess their experiences ? Allowing for the difficulty of putting precise words to inner experiences, it seemed from the informal interviews that educated marijuana smokers could give fairly articulate descriptions; and the respondents in this study were highly educated. Comprehensive interviews to determine a more adequate vocabulary are needed, but most of the possible effect descriptions seemed understandable and conveyed what the users wished to express. To check carelessness or deliberately bizarre answers, I added fourteen extra descriptions of possible effects randomly among the original 206. They constituted a validity scale by referring to unheard-of effects. Any questionnaire with six or more positive responses to them was to be rejected without further analysis; three were in fact rejected, leaving 150 that could be used.

A further objection: might the respondent overstress positive effects in order to justify his drug use? One cannot be certain, but two steps were taken to reduce the possibility. First, the accompanying letter was designed to appear sympathetic to marijuana use, so there was no need to win over the investigator. Second, the letter appealed to the respondents' curiosity about other users' experiences and to their loyalty to other users: because it promised to make the results available, the respondents would benefit by being accurate. Negative, unpleasant effects, are, nevertheless, under-represented in the replies, and interpretations should take account of the lack. By definition, an "experienced" marijuana user is an enthusiast; it would be somewhat perverse to indulge continually in criminal behaviour that is personally unpleasant.

Because my purpose is to ascertain the common effects of marijuana intoxication rather than the effects in specific populations, I describe here only major characteristics of the sample. The respondents were chiefly young Californians (67 per cent), with some East Coast respondents (11 per cent); 87 per cent were under 30 years old. There were twice as many males as females. 67 per cent were students, and 71 per cent unmarried. Only 7 per cent had had no college training, and 21 per cent had pursued graduate work or had earned advanced degrees. Only 4 per cent had been arrested for possessing marijuana. Many of the respondents were interested in self-improvement: 36 per cent reported that they practise some sort of discipline for spiritual or personal growth. 74 per cent of the sample had smoked marijuana for between six months and three years; the average frequency of use during the preceding six months ranged from "almost every day" (19 per cent) to "once a week or more" (42 per cent) to less than once a week (39 per cent). 72 per cent had tried more powerful psychoactive drugs such as LSD-25 at least once, 47 per cent of them in the six months covered by the questionnaire. Few (7 per cent) had used hard narcotics or dangerous stimulants (amphetamine or methedrine by injection). The respondents had used marijuana to alter their state of consciousness more than twice as frequently as alcohol in the preceding six months.

I classify the results into three categories: first, a large number of effects that may be considered common; second, a smaller number of effects that occur so frequently that they may be considered characteristic (these are shown by an asterisk in the list of "common effects"); third, some infrequent effects that seem at face value to be very significant. "Common" is here defined as being rated as "Sometimes", "Very Often", or "Usually" by at least 50 per cent of the respondents; "characteristic" as being rated "Very Often" or "Usually" by at least 50 per cent of the respondents.

The descriptions of possible effects within the "common effects" category are given in the wording of the questionnaire, except that the qualifying phrase "the effect is more pronounced during marijuana intoxication ['while stoned'] than normally" is usually omitted to save space, and some parts of the descriptions are omitted if the meaning is not thereby affected. The descriptions are put into sixteen classes, although some are relevant to more than one class. Within each class, the descriptions begin with the most common; the figures given are the total percentages of respondents putting an effect into the "Sometimes", "Very Often", or "Usually" categories.

Common Effects

Visual effects: (1) I can see patterns, form, figures, meaningful designs in visual material that does not have any particular form when I'm straight, that is just a meaningless series of lines or shapes when I'm straight, 85*. (2) If I try to visualize something . . . I see it in my mind's eye more sharply. . . , 81*. (3) When looking at pictures they may acquire an element of visual depth, a third dimensional aspect. . . , 72. (4) Things seen are seen more sharply in that their edges, contours stand out more sharply against the background, 72. (5) I can see new colours or more subtle shades of colour . . . , 70. (6) There is a sensual quality to vision, as if I were somehow "touching" the objects or people I am looking at, 59. (7) . . . things in the periphery of my vision look different when I'm not looking directly at them . . . , 58. (8) My visual perception of the space around me is changed so that what I'm looking at is very real and clear but everything else I'm not focusing on visually seems further away or otherwise less real or clear, 58.

Auditory effects: (1) I can hear more subtle changes in sounds, for example, the notes of music are purer and more distinct, the rhythm stands out more, 99*. (2) I can understand the words of songs which are not clear when straight, 85*. (3) When listening to stereo music or live music, the spatial separation between the various instruments sounds greater, as if they were physically further apart, 82*. (4) If I try to have an auditory image . . . it is more vivid . . . , 73. (5) With my eyes closed and just listening to sounds, the space around me becomes an *auditory* space, a space where things are arranged according to their sound characteristics instead of visual, geometrical characteristics, 65. (6) The sound quality of my voice changes, so that I sound different to myself when I talk, 63.

Touch effects: (1) My sense of touch is more exciting, more sensual . . . , 86*. (2) Touch sensations take on new qualities . . . , 85*. (3) Some surfaces feel much smoother, silkier, 77. (4) Some surfaces feel much rougher, irregular . . . and the roughness or graininess forms interesting patterns, 73. (5) The temperature of things . . . takes on new qualities, 69. (6) I can experience vivid tactual imagery . . . , 60. (7) Objects seem heavier, more massive when I lift them, 55.

Taste effects: (1) Taste sensations take on new qualities, 93*. (2) I enjoy eating very much and eat a lot, 93*. (3) If I try to imagine what something tastes like, I can do so very vividly, 69. (4) I crave sweet things to eat, like chocolate, more than other foods, 57.

Smell effects: (1) Smell sensations take on new qualities, 71. (2) Smells become richer and more unique . . . , 69.

Space-time perception: (1) When I walk someplace my experience of the distance covered is quite changed . . . , 96*. (2) Time passes very slowly . . . , 95*. (3) Distances between me and things or me and other people seem to get greater . . . , 69. (4) Events and thoughts flow more smoothly, the succession of events in time is smoother than usual, 69. (5) I get so lost in fantasy or similar trips in my head that I completely forget where I am, and it takes a while to reorient after I come back and open my eyes, 63. (6) Time seems to stop: it's not just that things take longer, certain experiences seem outside of time, are timeless, 63. (7) Events and thoughts follow each other jerkily, there are sudden changes from one thing to another, 59. (8) While something is happening I get the funny feeling that this sequence has happened before in exactly the same way . . . (*déjà vu*) . . . , 55. (9) Distances . . . seem to get shorter . . . , 53.

Perception of the body: (1) I feel a lot of pleasant warmth inside my body, 71. (2) If I am paying attention to some particular part of my body, the rest of my body fades away a lot so the part I'm attending to stands out more sharply, 69. (3) I am much more aware of the beating of my heart, 69. (4) With my eyes closed, my body may feel very light or even feel as if I float up into the air, 68. (5) I have lost all consciousness of my body during fantasy trips . . . , 66. (6) I get feelings in my body that are best described as energy, force, power of some sort flowing, 65. (7) I lose awareness of most of my body unless I specifically focus my attention there or some particularly strong stimulus demands my attention there, 61. (8) I become very aware of my breathing and can feel the breath flowing in and out of my throat as well as filling my lungs, 60. (9) Pain is easy to tolerate if I keep my attention elsewhere, 59. (10) I feel a vibration or tingling sensation in some or all of my body that I can tell is not an actual muscle tremor by looking at my body, 57. (11) Pain is more intense if I concentrate on it, 54.

Physical movement: (1) I get physically relaxed and don't want to get up or move around, 95*. (2) When I move about or dance my motions seem exceptionally smooth and well coordinated, 81*. (3) I get physically restless so that I want to move around a lot, 58. (4) I feel much weaker when stoned (regardless of whether you're actually physically stronger or weaker), 51.

Interpersonal relations: (1) I have feelings of deep insights into other people, how they tick, what their games are (regardless of whether they actually check out later), 85*. (2) I find it very hard to play ordinary social games, 83*. (3) I empathize tremendously with others, I feel what they feel, I have a tremendous intuitive understanding of what they're feeling, 83. (4) I talk a lot less, 83. (5) I am less noisy and boisterous at parties, 82*. (6) I am less noisy and boisterous at parties than when drunk or tipsy on alcohol, 82*. (7) When stoned with others I play "childish" games, that is, we interact with each other in ways which are very enjoyable but which people would ordinarily consider "childish", 80. (8) I feel the things I say in conversation are more profound and appropriate to the conversation, more interesting, 79. (9) I become more sociable, I want to be with and interact with people more, 76. (10) When stoned with a group of people, the group takes on a much greater sense of unity, or real social relationship . . . , 75. (11) I become less sociable; I want to be by myself, 73. (12) Being with people who are much higher than I am (as from their being on acid (LSD) or much more stoned on grass) gets me higher, even though I don't smoke any more grass, 70. (13) I talk a lot more, 64. (14) I feel isolated from things around me . . . , 50.

Sexual effects: (1) Sexual orgasm has new qualities, pleasurable qualities, 77*. (2) When making love I feel I'm in much closer mental contact with my partner; it's much more a union of souls as well as bodies, 76. (3) I have no increase in sexual feeling unless it's a situation that I would normally be sexually aroused in, and then the sexual feelings are much stronger and more enjoyable, 75. (4) My sexual drive goes up, I have more need for sex, 61. (5) I feel as if I'm a better person to make love with when stoned, 52.

Thought processes: (1) I appreciate very subtle humour in what my companions say, and say quite subtly funny things myself, 91*. (2) Commonplace sayings of conversations seem to have new meanings, more significance, 87. (3) I give little or no thought to the future, I am completely in the here-and-now, 87*. (4) Spontaneously, insights about myself, my personality, the games I play, come to mind when stoned and seem very meaningful, 86*. (5) The ideas that come to my mind are much more original, 83. (6) I find it difficult to read, 80*. (7) I think about things in ways that seem intuitively correct, but which do not follow the rules of logic, 78. (8) . . . I think much more in (visual) images instead of just abstract thought, 75. (9) I am more willing to accept contradictions between two ideas or two views . . . I don't get uptight because the two things don't make immediate sense, 74*. (10) If I deliberately work on it, I can have important insights about myself, my personality, the games I play, 73. (11) I learn a great deal about psychological processes . . . general knowledge about how the mind works (as opposed to specific insights about yourself), 71. (12) I get so wound up in thoughts or fantasies that I won't notice what's going on around me . . . , 69. (13) I get so wound up in thoughts or fantasies while doing some physical task or job that I lose awareness of doing it, yet suddenly find that I have finished the physical task . . . , 67. (14) I have more imagery than usual while reading; images of the scene I'm reading about just pop up vividly, 67. (15) I do things with much less thought to possible consequences of my actions . . . , 65. (16) If I try to solve a problem, it feels as if my mind is working much more efficiently than usual (regardless of how you evaluate the solution later), 64. (17) If I work on a problem, I work less accurately as judged by later real-world evaluation, 63. (18) I can play elaborate games and get very involved in the games, 60. (19) If I try to solve a problem it feels as if my mind is much less efficient . . . , 56. (20) In thinking about a problem of the sort that normally requires a series of steps to solve, I can get the answer without going through some of the usual intermediate steps . . . , 53.

Memory functioning: (1) My memory span for conversations is somewhat shortened, so that I may forget what the conversation is about even before it has ended (even though I may be able to recall it if I make a special effort), 89*. (2) I can continue to carry on an intelligent conversation even when my memory span is so short that I forget the beginnings of what I started to say; for example, I may logically complete a sentence even as I realize I've forgotten how it started, 72. (3) I can't think clearly, thoughts keep slipping away before I can quite grasp them, 71. (4) My memory span for conversations is very shortened so that I may forget what the start of a sentence was about even before the sentence is finished . . . , 68. (5) I spontaneously remember things I hadn't thought of in years . . . , 61. (6) If I read while stoned, I remember less of what I've read hours later, 61. (7) I think I've said something when actually I've only thought about saying it . . . , 57. (8) My memory of what went on while I was stoned is poor afterwards . . . , 57. (9) My memory of what went on is . . . better than if I had been straight, 55.

Emotions: (1) I feel emotions much more strongly, so they affect me more, 80. (2) I almost invariably feel good when I turn on, regardless of whether I felt bad before turning on, 80*. (3) I am more aware of the body tensions and feelings that are part of emotions, 74. (4) Whatever mood I was in before turning on becomes greatly amplified; so if I felt let down I really feel bad, and if I felt good, I really feel very good, 72.

Self-control: (1) I find it easy to accept whatever happens, I don't need to control it or feel in control of it, 89*. (2) I can "come down" at will if I need to be straight for a minute to deal with some complicated reality problem, 89*. (3) I often forget to finish some task I've started, or get sidetracked more frequently than when straight, 86*. (4) I giggle a lot when stoned, I am silly, even though the situation is not that funny, 74. (5) I have excellent control over my fantasies; I can make them go in whatever direction I want, 73. (6) My inhibitions are lowered so that I do things I'm normally too inhibited to do (Note: this does not apply to antisocial acts but to acts that are generally acceptable but that you normally can't do through shyness or the like), 69. (The immediately following item was, "I lose control of my actions and do antisocial things [actions that harm other people] that I wouldn't normally do." The response was 77 per cent never, 22 per cent rarely, and one respondent sometimes.) (7) I can work at a necessary task with extra energy, absorption and efficiency, 61. (8) I feel as if I lose control over my thoughts; they just go on regardless of what I want (without reference to whether you like this or not), 52.

Sense of identity: (1) I feel very powerful, capable, and intelligent, 71. (2) Some events become archetypal, part of the basic way Man has always done things. That is, instead of me (John Doe, ego) doing something, it is just Man Doing What Man Has Always Done . . . , 57. (3) I lose all sense of self, of being a separate ego, and feel at one with the world, 55.

Effects on sleep: (1) I find it very easy to go to sleep at my usual bedtime when stoned, 84*. (2) I get very drowsy even though it's not late . . . , 83. (3) My sleep is particularly refreshing if I go to bed stoned, 81. (4) My dreams are more vivid if I go to bed stoned, 51.

Miscellaneous effects: (1) I feel more childlike, more open to experiences of all kinds, more filled with wonder and awe at the nature of things, 91*. (2) I get more involved in ordinary tasks, 80. (3) Others (who were straight at the time) have not noticed that I've been stoned (applied to other people who were your friends and would have told you if they'd noticed), 69. (4) With my eyes closed my inner visions and fantasies become extremely real, as real as night-time dreams, 68. (5) Some of my inner trips, eyes-closed fantasies, have been so vivid and real that even though I know logically they couldn't be real, they feel real; they are as real as ordinary waking life experience, 57. (6) Sounds have visual images or colours associated with them, synchronized with them, 55.

Infrequent but Significant Effects

The following effects were not reported often enough to be classed as common, but they seem so powerful and unusual that they might well change somebody's beliefs. The numbers given are the percentages of respondents who have experienced the effect at all.

(1) I feel so aware of what people are thinking that it must be telepathy, mind reading, rather than just being more sensitive to the subtle cues in their behaviour, 69; (2) Getting stoned has acquired a religious significance for me, 69; (3) I have spiritual experiences . . . which have had a powerful, long-term religious effect on me, 65; (4) I feel in touch with a Higher Power or a Divine Being to some extent . . . , I feel more in contact with the "spiritual" side of things, 59; (5) I am able to meditate more effectively, 39; (6) I can foretell the future by some kind of precognition, more than just logically predicting from present events, 32; (7) . . . felt "located" outside the physical body . . . , 32; (8) I can perform magical operations that will affect objects or people, 13.

The way in which items were selected for the questionnaire (informal interviewing) produced a set of items that were almost uniformly positive or neutral in tone; there were almost no "bad" effects. But a few negative effects included in the questionnaire are powerful enough to seem important, even if they are infrequent. Figures are again the percentages of respondents who have experienced the effect at all.

(9) I get somewhat paranoid about the people with me, I am suspicious about what they're doing, 80; (10) I lose control of my actions and do antisocial things (things that harm other people), 23; (11) I have lost control and been "taken over" by an outside force or will which is hostile or evil in intent for a while, 20.

One question asked the respondents how often they had seen other people "freak out", that is, have intense, transient emotional upsets. 62 per cent of the respondents said never, 36 per cent less than one time in twenty, and 2 per cent more often. Fifty-three respondents answered a question about what sort of help was necessary for the person who "freaked out"; usually (64 per cent) friends talked to the person and calmed him. Medical or psychological aid was used in 13 per cent of cases, with other methods or no special help for the rest. When the respondents were asked how many times they had "freaked out" themselves, 79 per cent answered never, 14 per cent once, and 7 per cent more than once. The respondents themselves were usually calmed down by friends (56 per cent) or had the "freakout" subside by itself or through their own efforts (37 per cent).

These figures probably represent a higher incidence of "freakouts" on marijuana than actually occurs: because of the method of distributing questionnaires, many of the respondents may have been reporting on the same cases of emotional difficulties in others.

Pleasures of Being High

124 of the 206 items on the questionnaire may thus be considered common experiential effects of marijuana intoxication. Sense perception is often improved, both in intensity and in scope. Imagery is usually stronger but well controlled, although people often care less about controlling their actions. Great changes in perception of space and time are common, as are changes in psychological processes such as understanding, memory, emotion, and sense of identity.

This is not the place to theorize about the results, but they are quite consistent with an earlier, independent description: " . . . Sensations are enhanced and clarified: sight, hearing, taste, touch. Time perception changes. Attention becomes more unified, and moves more into preconscious material and the state of pure awareness. The many broad processes of association, such as social meanings, memory images, expectancies and plans, are reduced in number and relevance. Inhibitions and suppressions relax, allowing emotions, thoughts, fantasies, and memories to flow more freely. The development and strength of these effects will depend on the individual, the times he has used marijuana, how he has used marijuana, and the environment"[11]. Although the validity of the descriptions cannot be proved, there is at least a great deal of agreement among the respondents. To the extent that the described effects are delusory or inaccurate, the delusions and inaccuracy are widely shared. It is interesting, too, that nearly all the common effects seem either emotionally pleasing or cognitively interesting, and it is easy to see why marijuana users find the effects desirable regardless of what happens to their external behaviour. But it should be remembered that negative effects are probably somewhat under-represented.

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Specificity in Bacterial Protein Synthesis: Role of Initiation Factors and Ribosomal Subunits

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Ribosomes from *E. coli* initiate synthesis *in vitro* of all three of the proteins coded by phage f2, but ribosomes from *B. stearothermophilus* initiate synthesis of only one. The specificity of initiation depends only on the source of the 30S subunits; the origin of the 50S subunits and initiation factors has no effect.

THE initiation of protein synthesis in bacteria involves two ribosome subunits, messenger RNA containing an AUG or GUG initiator codon, GTP, N-formylmethionyl-tRNA, and at least three protein initiation factors[1-7]. Most AUG or GUG sequences on a messenger, however, do not normally act as signals for polypeptide chain initiation; rather they code for an internal amino-acid or they are out of phase with the normal reading frame. The complex of ribosomes and initiation factors (which normally are isolated together) plays a critical part in recognizing the proper AUG (or GUG) sequences on a messenger as signals for initiation[8,9]. Addition of RNA from bacteriophage f2 to a cell-free system containing the ribosome–factor complex from its host cell, *Escherichia coli*, results in synthesis of all three f2 proteins: coat protein, RNA replicase and maturation protein[8,10-13]. By contrast, ribosomes from *Bacillus stearothermophilus* direct synthesis only of the maturation protein, the product made in least amounts by *E. coli* ribosomes. The origin of transfer RNA or supernatant enzymes had no effect on the product formed[8,9]. Direct experiments have confirmed that *B. stearothermophilus* ribosomes bind only to the sequence on f2 RNA which codes for the amino terminus of the maturation protein[13].

Initiation factors are required for translation only of messenger RNAs which contain an AUG or GUG initiator codon[1,3-5]. One factor (B or f3) is required for binding ribosomes to natural messenger RNA, but not to certain synthetic messenger RNAs which contain an AUG initiation sequence[5,14,15]. This suggests that the initiation factors serve to recognize the proper initiation sequences on a natural messenger RNA. We show here, however, by experiments which mix components of the *E. coli* and *B. stearothermophilus* systems, that it is the origin of the 30S ribosome subunits which determines which f2 proteins are produced; the species from which the initiation factors or 50S subunits are isolated is irrelevant. Thus the 30S subunit selects regions of messengers as signals for the initiation of polypeptide synthesis; the three initiation factors and the 50S subunit, while essential for translation of natural messenger RNA, appear to have no function in this process.

Lack of Specificity of Initiation Factors

Ribosomes and initiation factors were prepared from both *E. coli* and *B. stearothermophilus* extracts by ultracentrifugation in 2 M NH_4Cl solutions (see legend to Table 1). In the absence of initiation factors, both types of ribosomes are completely inactive in directing protein synthesis with either f2 RNA (Fig. 1, Table 1), *E. coli* mRNA, or T4 mRNA (unpublished). By contrast, with poly U as messenger, both ribosomes had the same high activity in directing synthesis of polyphenylalanine, and addition of either preparation of initiation factors had no effect (less than ±20 per cent) (unpublished). Using f2 RNA, ribosomes from either species were stimulated by either factor preparation. Regardless of the factor used, *E. coli* ribosomes synthesized 15–20 times as much f2 protein as did *B. stearothermophilus* ribosomes.

To determine which f2 proteins were synthesized by the different ribosome-factor mixtures, we identified their N-termini by their content of N-formylmethionine. f2 specific proteins synthesized *in vitro* and labelled with ^{35}S fMet-tRNA were digested with trypsin and chymotrypsin and subjected to paper chromatography and ionophoresis at *p*H 3·5 as described previously[8-10,16]. Table 1 shows that addition to *B. stearothermophilus* ribosomes of either *B. stearothermophilus* or *E. coli* initiation factors results in synthesis of only the f2 maturation protein. Conversely, addition of either preparation of factors to *E. coli* ribosomes results in normal synthesis of all three f2 proteins. A control experiment (last line, Table 1) shows that *B. stearothermophilus* ribosomes contain nothing which might destroy either the capacity of f2 RNA to synthesize coat protein or any coat or RNA replicase produced[8].

These results mean that the nature of proteins synthesized is determined solely by the nature of the ribosomes; the initiation factor preparations do not, in this system at least, contribute to the specificity of protein synthesis. In particular, *B. stearothermophilus* initiation factors allow *E. coli* ribosomes to synthesize both the f2 coat and RNA replicase proteins even though the mixture

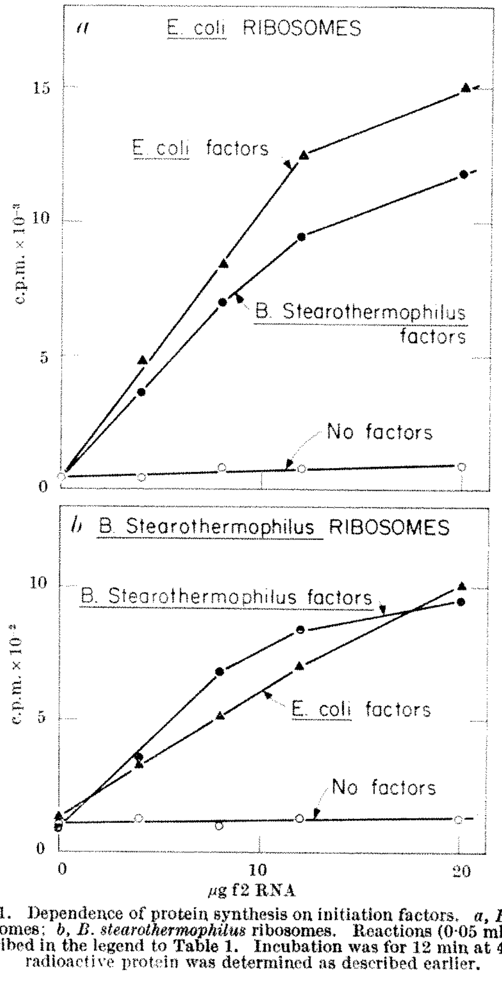

Fig. 1. Dependence of protein synthesis on initiation factors. *a*, *E. coli* ribosomes; *b*, *B. stearothermophilus* ribosomes. Reactions (0·05 ml.) are described in the legend to Table 1. Incubation was for 12 min at 47° C; radioactive protein was determined as described earlier.

Table 1. EFFECT OF INITIATION FACTORS ON PROTEIN SYNTHESIS BY f2 RNA

Ribosomes	Factors	RNA	c.p.m. reaction Coat	Poly-merase	Matura-tion
E. coli	0	0	(56)	(65)	(51)
		f2	420	200	17
	E. coli	0	(61)	(38)	(66)
		f2	46,864	24,648	1,989
	B. stearothermophilus	0	(63)	(54)	(39)
		f2	35,526	21,435	1,632
B. stearothermophilus	0	0	(41)	(31)	(49)
		f2	43	15	−6
	E. coli	0	(89)	(74)	(41)
		f2	14	26	3,480
	B. stearothermophilus	0	(115)	(81)	(77)
		f2	−7	51	4,320
E. coli plus *B. stearothermophilus*	*E. coli*	0	(79)	(133)	(120)
		f2	44,920	27,100	2,940

Reactions (0·30 ml.) contained, per ml.: 50 μmoles ammonium cacodylate (pH 7·0), 100 μmoles NH_4Cl, 9 μmoles magnesium acetate, 10 μmoles 2-mercaptoethanol, 2 μmoles ATP, 4·2 μmoles PEP, 0·3 μmoles GTP, 0·1 μmoles each of twenty non-radioactive amino-acids; 500 μg *E. coli* tRNA charged only with ^{35}S-N-formylmethionine[16] (5,500 mCi/mmole), 0·20 ml. S100 from *B. stearothermophilus*; and, where indicated, 200 μg f2 RNA; 40 $A_{260\ nm}$ units *E. coli* ribosomes, 56 $A_{260\ nm}$ units *B. stearothermophilus* ribosomes; 280 μg crude *E. coli* initiation factors; and 360 μg crude *B. stearothermophilus* initiation factors. Incubation was at 47° C for 12 min. Isolation of the radioactive protein, digestion with trypsin and chymotrypsin, and separation and quantitation of the amino terminal peptides of the phage proteins have been described previously[8,16,25]. Backgrounds from samples without f2 RNA have been subtracted from the f2 samples. Ribosomes and initiation factors were prepared by a modification of the technique of Anderson *et al.*[1]. Frozen *E. coli* PR7 or *B. stearothermophilus* strain 10 log-phase cells[18] were ground with 2·5 weights of alumina, extracted with 3 volumes of 0·03 M Tris (pH 7·8), 0·06 M KCl, 0·01 M magnesium acetate, 0·003 M 2-mercaptoethanol (buffer A) containing 1 μg/ml. deoxyribonuclease and centrifuged twice for 20 min at 20,000g. To the supernatant ATP and PEP (final concentrations 0·0025 M and 0·0035 M, respectively) were added, and the mixture was preincubated 1 h at 30° C (*E. coli*) or 55° C (*B. stearothermophilus*). Careful control experiments showed the preincubation resulted in no loss of the ability to support f2 or T4 RNA directed protein synthesis. The extract was centrifuged 4·5 h at 40,000 r.p.m. in the Spinco '40' or '65' rotor; the ribosomal pellet was resuspended in buffer A, clarified by low speed centrifugation and pelleted again in the ultracentrifuge. The pellet was resuspended overnight in 2·0 M NH_4Cl, 0·05 M Tris (pH 7·8), 0·01 M magnesium acetate, 0·006 M mercaptoethanol (buffer B) and then the ribosomes were sedimented in the ultracentrifuge for 5 to 6 h at 45,000 r.p.m. Initiation factors were prepared from the top half of the supernatant by $(NH_4)_2SO_4$ precipitation[1]. The ribosomal pellet was resuspended again overnight in buffer B, recentrifuged, resuspended in buffer A, and stored frozen in small aliquots at −80° C.

of *B. stearothermophilus* ribosomes and factors synthesizes neither.

If supernatant enzymes are omitted, this system can form only the first peptide bond of the f2 proteins. After alkaline hydrolysis of the resultant dipeptidyl-tRNA linkage, the ^{35}S peptides can be analysed by paper ionophoresis at pH 3·5. Without added viral RNA (Fig. 2*b*, *d*), the only products are radioactive methionine, formylmethionine, and formylmethionine sulphoxide, all of which are derived from the tRNA added to the reaction[17]. Addition of f2 RNA to *E. coli* ribosomes and factors results in formation of two new radioactive peptides, I and II (Fig. 2*c*). Peptide I is N-formylmethionylalanine, the dipeptide at the amino terminus of the coat protein[17]. Peptide II is N-formylmethionylarginine, the dipeptide found at the amino terminus of the maturation protein[8,9]. These results show that *E. coli* ribosomes direct synthesis of the first peptide bond of both the f2 coat and maturation proteins[17]. No N-formylmethionylserine, the amino terminal peptide of the RNA replicase, is seen[16], suggesting that ribosomes normally cannot directly initiate synthesis of this protein[16–18].

By contrast, *B. stearothermophilus* ribosomes and initiation factors only direct synthesis of peptide II, from the maturation protein, and no peptide I from the coat (Fig. 2*a*, Table 2). Control experiments (Table 2), in which *E. coli* and *B. stearothermophilus* ribosomes were mixed, showed that the latter contain no inhibitor for the synthesis of fMet-Ala. Table 2 shows that formation of any formylmethionyl dipeptide by ribosomes from either species is dependent on addition of initiation factors. Addition of either *E. coli* or *B. stearothermophilus* factors to *E. coli* ribosomes permits normal formation of the first peptide bond of both coat and maturation protein. Also, addition of either factor preparation to *B. stearothermophilus* ribosomes permits initiation only of the maturation protein. Again, the nature of the proteins initiated is determined only by the origin of the ribosomes, and not of the initiation factors.

Role of Ribosome Subunits

Table 3 shows that addition of purified *E. coli* 30S subunits to an f2 RNA primed *B. stearothermophilus* cell-free system causes the synthesis of high levels of both f2 coat protein and RNA replicase protein (in addition to the normal synthesis of maturation protein). Addition of an equivalent amount of unfractionated *E. coli* ribosomes (30S + 50S) has the same effect, while addition of purified *E. coli* 50S subunits induces little synthesis of either coat or replicase. The small synthesis of these proteins can be accounted for by the 7 per cent contamination of the 50S subunit preparation by 30S subunits (see Table 3). This experiment shows that the *E. coli* 30S subunit, and not the 50S subunit, is responsible for recognition of the regions of f2 RNA which code for initiation of coat and replicase synthesis.

Thus the nature of proteins synthesized by f2 RNA is determined only by the origin of the 30S ribosomal subunit; 30S ribosome subunits from *E. coli* direct synthesis of all three f2 proteins, while *B. stearothermophilus* 30S subunits direct synthesis of only the f2 maturation protein. This effect is independent of the origin of the 50S subunits (Table 3), initiation factors (Tables 1, 2), transfer RNA, or supernatant enzymes. Ribosomes from the mesophile *B. megaterium* translate the same f2 protein as do those from *B. stearothermophilus* (unpublished results of T. Johnson and H. F. L.).

We have also studied messenger RNA from growing *E. coli* cells, and from cells 5 and 15 min after infection by T4 phage. In an *E. coli* cell-free system all three messengers induced the synthesis of 15–20 new proteins, as shown by peptide mapping of protein labelled by ^{35}S-fMet-tRNA. *B. stearothermophilus* extracts synthesized only about one-fourth of the *E. coli* mRNA directed proteins and two-thirds of the T4-specific proteins. Mixing experiments analogous to those reported here on f2 RNA showed that the nature of the proteins produced depended on the origin of the ribosome subunits, and not on the species from which initiation factors were isolated.

Table 2. SYNTHESIS OF FIRST PEPTIDE BOND OF COAT AND MATURATION PROTEIN

Ribosomes	Factors	f2 RNA	Peak I fMet-Ala (coat)	Peak II fMet-Arg (maturation)
E. coli	0	0	(116)	(114)
		f2	−21	−24
	E. coli	0	(206)	(160)
		f2	1,902	636
	B. stearothermophilus	0	(149)	(120)
		f2	1,310	440
B. stearothermophilus	0	0	(89)	(101)
		f2	−2	2
	E. coli	0	(116)	(118)
		f2	−33	717
	B. stearothermophilus	0	(139)	(149)
		f2	−41	641
E. coli plus *B. stearothermophilus*	*B. stearothermophilus*	0	(113)	(116)
		f2	914	540

Reactions (0·3 ml.) were as described in the legend to Fig. 2, except that ribosomes, washed in 2 M NH_4Cl solution, and initiation factors were used at the concentrations described in the legend to Table 1. Analysis of the reaction products is described in the legend to Fig. 2. Backgrounds from reactions without f2 RNA (in parentheses) have been subtracted from the values from the f2 reactions.

Table 3. EFFECT OF ADDITION OF *E. coli* SUBUNITS

Additions	c.p.m./reaction Coat	Polymerase	Maturation
(No RNA)	(160)	(123)	(229)
f2 RNA	116	195	4,618
f2 RNA plus 11 *A* units *E. coli* 30S + 50S	27,728	6,492	2,791
f2 RNA plus 5·1 *A* units *E. coli* 30S	18,857	4,080	2,060
f2 RNA plus 7·4 *A* units *E. coli* 50S	2,767	718	2,066

Reactions (0·5 ml.) were as described in the legend to Table 1, except 0·30 ml. of preincubated *B. stearothermophilus* S-30[16] was used in place of S100 and *B. stearothermophilus* ribosomes. *E. coli* ribosomes, washed twice in buffer A, were separated into subunits by dialysis overnight in 0·03 M Tris (pH 7·8), 0·1 M NH_4Cl, 0·0005 M magnesium acetate, 0·003 M 2-mercaptoethanol (buffer C) and centrifugation in a 15–30 per cent sucrose gradient in buffer C ('SW 27' rotor in Beckman 'L2–65B' ultracentrifuge). To the 30S and 50S peak tubes was added magnesium acetate to 0·01 M, and then three volumes of buffer A; the subunits were pelleted by ultracentrifugation, resuspended in buffer A, run through a second sucrose gradient (5–20 per cent in buffer A), then pelleted as above, and resuspended in buffer A. On analysis in small sucrose gradients (12 ml. 5 to 20 per cent gradients in buffer A, 'SW 36' rotor), the 30S subunits contained no detectable 50S, while in the 50S preparation 5 to 8 per cent of the $A_{260\ nm}$ was in 30S subunits. An aliquot of the ribosomes just before layering on the first sucrose gradient was taken, made 10^{-2} M in Mg^{2+}, and used at the "30S + 50S" preparation (line 3).

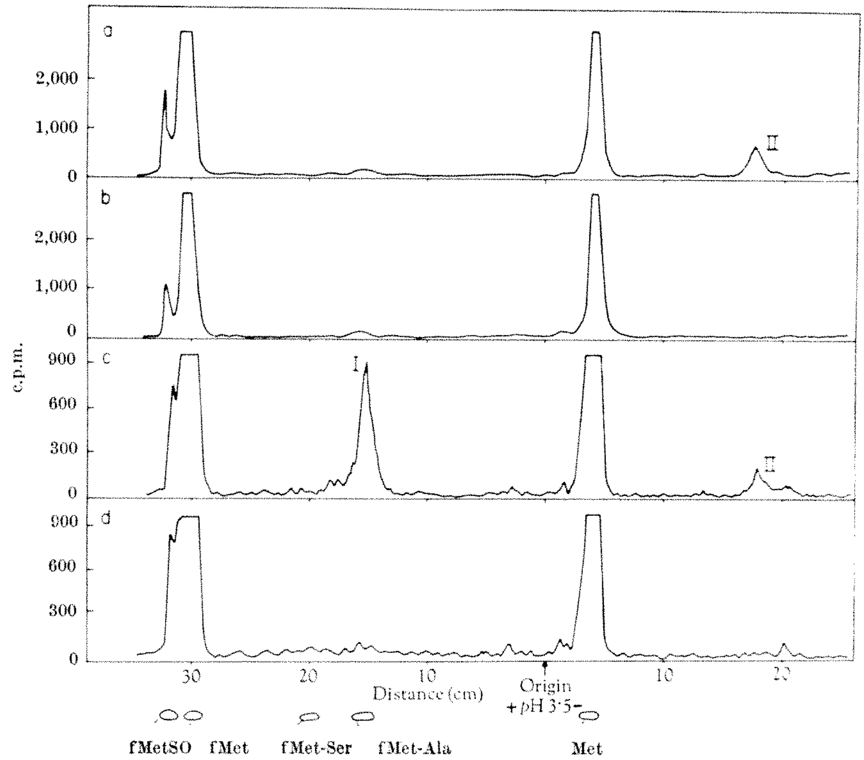

Fig. 2. Synthesis of first peptide bond of f2 proteins by *E. coli* and *B. stearothermophilus* ribosomes. *a*, *B. stearothermophilus* ribosomes plus f2 RNA; *b*, *B. stearothermophilus* ribosomes; *c*, *E. coli* ribosomes plus f2 RNA; *d*, *E. coli* ribosomes. Reactions[17] (0·1 ml.) contained, per ml., 100 µmoles HEPES (pH 7·0), 50 µmoles KCl, 8 µmoles magnesium acetate, 0·3 µmoles GTP, 500 µg *E. coli* tRNA charged with ^{35}S-methionine (5,600 mCi/mmole, about 70 per cent of methionine tRNA formylated) and nineteen non-radioactive amino-acids, 38 $A_{260\ \text{nm}}$ units of *E. coli* ribosomes or 50 $A_{260\ \text{nm}}$ units *B. stearothermophilus* ribosomes. Both ribosome preparations were washed three times in buffer A only, and retained all of the initiation factors. Incubation was for 10 min at 47° C; 0·5 ml. 0·4 M triethylamine was added and the reaction incubated at 37° C for 1 h. After lyophilization, the samples—together with marker peptides—were analysed by paper ionophoresis at pH 3·5. Radioactivity was measured in a Packard '7201' strip scanner at 0·2 cm/min, with a 1,000 c.p.m. scale[17].

B. stearothermophilus and *E. coli* factors stimulated *E. coli* ribosomes to make all of the *E. coli* and T4-specific proteins, while *E. coli* factors could not induce *B. stearothermophilus* ribosomes to synthesize the proteins not usually made. We shall report these experiments elsewhere.

The first step in the initiation of protein synthesis is the interaction of the 30S ribosome subunit, messenger RNA, and two initiation factors, B (f3) and C (f2). The initiator, fMet-tRNA, and the third factor, A (f1), are added, and finally the 50S subunit and the second aminoacyl-tRNA[1-5,19,20]. The work of Steitz[13] and Hindley[21] suggests that the site on the messenger to which the 30S subunit attaches is identical to the site at which protein synthesis is initiated. At least two considerations seem to determine which of many AUG or GUG sequences on a messenger serve as initiation signals.

First, the secondary structure of the messenger may restrict certain AUG or GUG codons from acting as initiator sequences. As shown by sequence analysis of parts of the phage RNA, many non-initiating AUG sequences seem to be in small double-stranded regions[13,21-23]. Moreover, mild disruption of f2 RNA by reaction with formaldehyde allows the RNA to initiate at least three new polypeptides which do not correspond to any known f2 protein[9,18]. This implies that in f2 RNA there are AUG sequences which can initiate protein synthesis, but are prevented by the RNA structure from doing so.

Second, our experiments show that the 30S subunit recognizes only some of the "exposed" AUG sequences as actual initiation signals. We suggest that the initiation factors, particularly factor B (f3)[3,14,24], do not play any part in messenger recognition. If factor B were to play such a part, it should comprise two or more components to enable it to recognize different initiation signals on different messengers; no such separation has been reported.

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Excision-repair Properties of an *Escherichia coli* Mutant deficient in DNA Polymerase

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An *Escherichia coli* mutant, extracts of which show no DNA polymerase activity, can excise pyrimidine dimers induced by ultraviolet irradiation. The increased radiation sensitivity of this strain is related to the extensive degradation of irradiated DNA.

An excision-repair mechanism for correcting structural distortions of the Watson–Crick helical structure of DNA was proposed in 1964[1,2]. An extensively used system for investigating the proposed mechanism has been DNA that contains cyclobutane-type pyrimidine dimers produced by irradiation with ultraviolet light (UV). Recent studies with purified DNA polymerase from *Escherichia coli* have suggested that this enzyme may play an important part in excision. The homogeneous protein preparation used in these studies, besides possessing DNA polymerase activity, had other catalytic properties, all contained in a single polypeptide chain of 109,000 molecular weight[3]. Kelly *et al.*[4] have shown that *in vitro* the purified preparation exhibits both polymerase and exonuclease activity on UV-irradiated DNA in which nicks were introduced with pancreatic deoxyribonuclease. The result of these activities is that the nicks are translated in the 5′→3′ direction and oligonucleotides that contain dimers are excised. Thus the exonuclease has the special function of producing the nick which is required for removal of the oligonucleotide containing the dimer.

As suggested by Kelly *et al.*[4], the "role of DNA polymerase (*in vivo*) would best be demonstrated if a mutant in repair or recombination had a defect in its DNA polymerase". De Lucia and Cairns[5] have isolated a mutant (designated P3478) and identified it not by a defect repair but by the inability of mutant cell extracts to incorporate labelled deoxyribonucleoside triphosphates into DNA. This mutant, which grows normally and has nearly normal recombination ability, is sensitive to inactivation by UV and methyl methane sulphonate[5,6]. We wish to describe the results of a series of experiments designed to determine the cause of the increased UV sensitivity of the mutant.

Cells were grown with aeration to 2×10^8/ml. in tryptone broth (NaCl, 5·0 g; Bacto tryptone, 5·0 g; nutrient broth, 3·0 g; maltose, 2·0 g; H_2O, 1 l.) supplemented with 2 μg/ml. thymidine. Radioactive labelling, when required, was achieved by adding 10 μCi/ml. ^{3}H-thymidine (6·7 Ci per mmole) or 0·5 μCi/ml. ^{14}C-thymidine (59 mCi per mmole). (Both radiochemicals were obtained from New England Nuclear Corp.) Biological assays were made on tryptone agar (NaCl, 5·0 g; Bacto tryptone, 5·0 g; Bacto peptone, 5·0 g; Bacto agar, 12·0 g; H_2O, 1 l.) supplemented with 2 μg/ml. thymine and 1 μg/ml. thiamine. Cultures to be irradiated with UV were centrifuged, washed and resuspended in buffer (Na_2HPO_4, 0·12 g; KH_2PO_4, 0·6 g; NaCl, 0·01 g; NH_4Cl, 0·2 g; $MgSO_4$, 4·54 g; gelatin, 0·1 g; H_2O, 1 l., *p*H 7·0). For irradiation of large numbers of cells, stirred suspensions at 1×10^9 cells per ml. in buffer were irradiated in a thin-layer under a germicidal lamp. The effective UV dose was calibrated retrospectively from the survival of the cells compared with a survival curve obtained in identical conditions with a transparent cell suspension.

DNA synthesis and degradation were measured in terms of changes in acid-insoluble radioactivity. 100 μl. volumes of ^{3}H or ^{14}C-thymidine labelled cell cultures were pipetted on to Whatman No. 3 filter paper disks, which were then washed in ice-cold 5 per cent trichloroacetic acid (TCA), rinsed with ethanol, and dried at 80° C. Radioactivity was counted with a toluene–BBOT counting solution and a Packard 'Tri-Carb' scintillation counter.

Methods for measuring dimer excision and the production of single-strand breaks in irradiated DNA have been described previously[1,7,8]. To limit extensive degradation of the DNA of UV-irradiated P3478 cells before they were ready to be handled, we irradiated the cells in chilled buffer containing 10^{-2} M KCN. The cyanide was removed by centrifugation and dilution of the cells into warm broth at zero time.

The Fate of DNA in Irradiated Cells

The mutant strain is about five times as sensitive as its parent to UV inactivation (Fig. 1). In our conditions the 1/*e* doses taken from the final slopes of the survival curves were 20 and 100 ergs/mm² for P3478 and W3110, respectively.

When cells prelabelled with ^{14}C-thymidine are washed, irradiated with UV, and incubated in growth medium

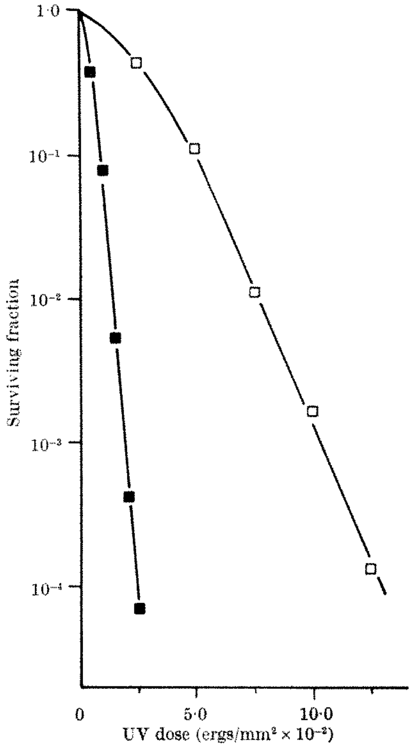

Fig. 1. UV survival curves. Stirred suspensions of cells at 2×10^7 per ml. in buffer were irradiated with germicidal light at 254 nm. □, W3110; ■, P3478.

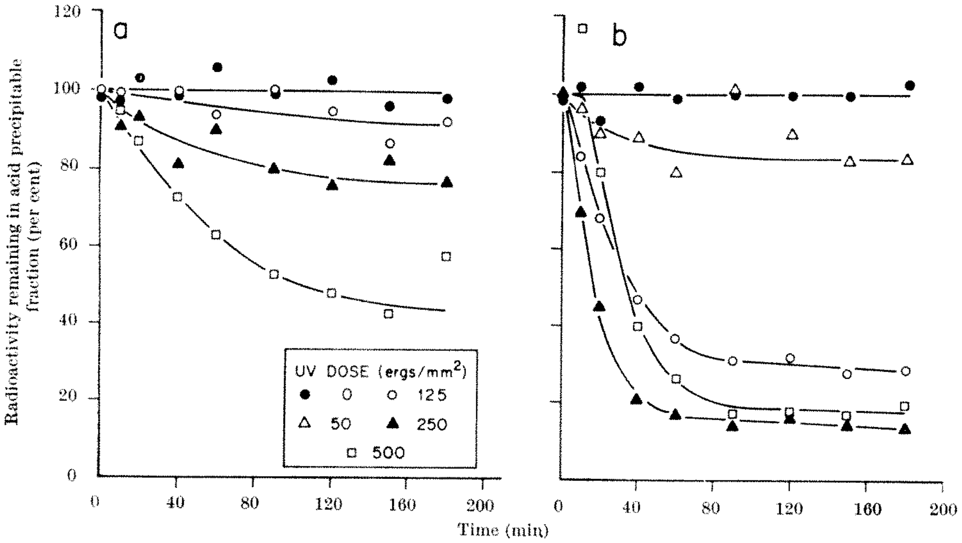

Fig. 2. Degradation of UV-irradiated DNA. Cells which had grown for several generations in tryptone broth + 2 μg per unlabelled thymidine + 0·5 μCi/ml. ^{14}C-thymidine were centrifuged, washed and resuspended in buffer at 2×10^7 per ml. Cells were irradiated with the indicated doses at 265 nm and then diluted into tryptone broth + 2 μg per ml. unlabelled thymidine at 37° C. At intervals, samples were taken and the TCA-precipitable radioactivity was counted. *a*, W3110; *b*, P3478.

containing 2 μg/ml. unlabelled thymidine, it is possible to detect degradation of the DNA (Fig. 2). The high sensitivity of the mutant cells (Fig. 1) is clearly related to the extensive degradation of DNA into TCA-soluble components at low doses (Fig. 2*b*). The DNA of P3478 is degraded faster and more extensively than W3110 DNA after irradiation with the same UV dose. Consequently, incorporation of exogenous label into DNA should be inhibited to a greater extent in P3478 than in W3110, because in the mutant cell population there would be not only fewer intact templates to replicate but endogenous label from breakdown would tend to be incorporated first. The data in Fig. 3 confirm this. Comparison of Figs. 1, 2 and 3 shows that UV doses which produce similar survivals generally cause similar extents of degradation and inhibition of post-UV DNA synthesis. This correlation does not seem to hold for the smallest UV doses; for example, 50 ergs/mm² has a similar effect on DNA synthesis for both strains.

The Excision of Dimers

We determined whether UV-induced pyrimidine dimers remained acid-insoluble following exposure of cells to small UV doses. In the experiments shown in Fig. 4, UV doses from 70–180 ergs/mm² converted 0·03–0·08 per cent of the radioactivity in thymine to thymine-containing dimers, which corresponds to between 300 and 770 dimers per DNA molecule[7]. The initial rate of dimer excision by P3478 is about half that of W3110 but continues longer in P3478, so that by 40 min only slightly fewer dimers have been excised. It is difficult to judge whether these differences in excision kinetics are significant or whether they result from the more extensive degradation of DNA by P3478 seen in Fig. 2. Table 1 is a balance sheet of the radioactivity for experiment 2 of Fig. 4. About 70 per cent of the radioactivity is accounted for, and as dimers are removed from the TCA-insoluble fraction they increase in the TCA-soluble fraction.

Two aspects of these experiments are unusual for the excision of dimers in *E. coli*. A delay of some 5 min is observed before excision commences (Fig. 4) and about 20 per cent of the total radioactivity appears in the growth medium of both cultures after 40 min of incubation (Table 1). Approximately one-half of the radioactivity from W3110 medium and one-third of that from P3478 medium was probably in the form of polynucleotides, because this material was insoluble in 5 per cent TCA and remained near the origin when chromatographed with a butanol–acetic acid–water (80 : 12 : 30) solvent. The remaining activity moved to the position of thymine or thymidine. We conclude that a sizable fraction of the radioactivity in the medium is DNA, probably from cells which have lysed, and presume that both the delay in excision and the lysis of cells occurred as a result of irradiating the cells in the presence of 10^{-2} M KCN.

Clearly the five-fold increase in UV sensitivity of the mutant cells is not the result of a failure to excise dimers, because this defect at most would account for only a two-fold increase in sensitivity. Rather, the increased sensitivity seems to be better explained by the correlated increase in nuclease activity. Alkaline sucrose gradient analyses showed that the average molecular weight of the DNA of UV-irradiated mutant cells was always larger than that expected if one single strand break per dimer was produced. This indicates that DNA degradation in these cells occurred as a result of exonuclease activity, rather than that of a rampant endonuclease. The same data (to be published elsewhere) suggested that although the more extensive degradation of the DNA

Table 1. BALANCE SHEET FOR EXCISION OF PYRIMIDINE DIMERS

Strain	Incubation (min)	Radioactivity in cells				Incubation medium (c.p.m. × 10^{-4})	Total radio-activity (c.p.m. × 10^{-4})	Calculated total c.p.m. for dimers in cells
		TCA insoluble		TCA soluble				
		Thymine (c.p.m. × 10^{-4})	Dimer (c.p.m.)	Thymine (c.p.m. × 10^{-4})	Dimer (c.p.m.)			
W3110	0	35	200	2·0	5·5	—	37	206
	10	20·8	35	0·34	69	2·0	23	104
	25	16·4	16	0·79	71	3·3	20	87
	40	16·3	35	1·3	140	5·4	23	175
P3478	0	36·9	138	2·3	3·3	—	39	141
	10	30·3	101	2·3	51	2·1	35	152
	25	25·8	35	0·77	72	4·5	31	107
	40	20·9	37	0·93	123	6·2	28	160

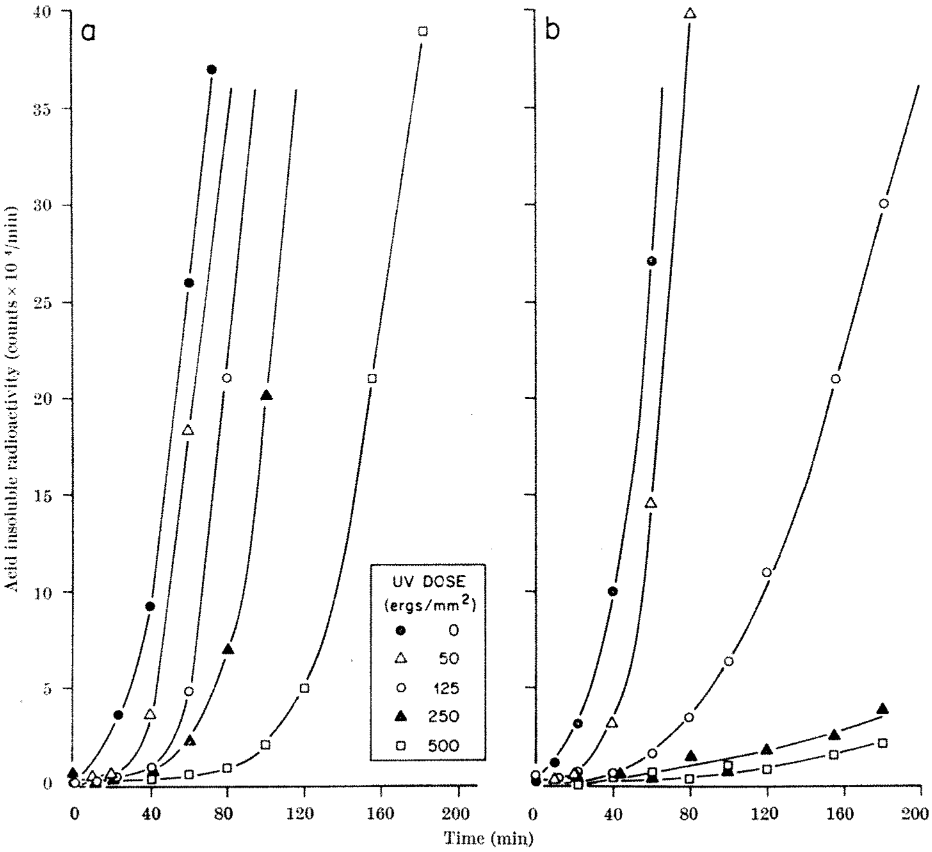

Fig. 3. Post-UV DNA synthesis. Non-radioactive cells were grown and irradiated as for Fig. 2. After irradiation, cell samples were diluted in tryptone broth + 2 μg per ml. unlabelled thymidine + 100 μCi/ml. ^{3}H-thymidine. At intervals, samples were taken and treated as in Fig. 2. *a*, W3110; *b*, P3478.

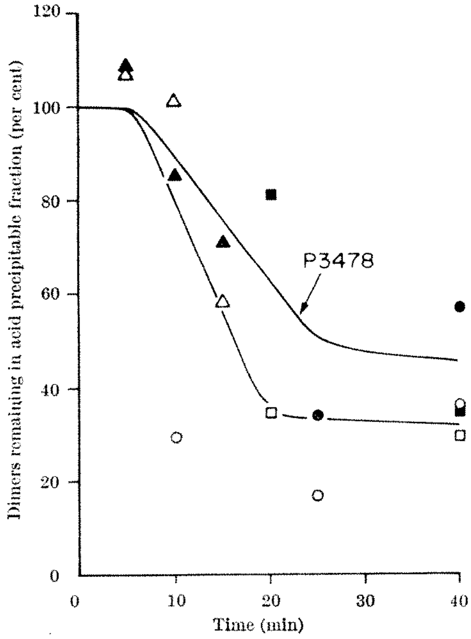

Fig. 4. Rate of dimer excision. Cells labelled with ^{3}H-thymidine were irradiated at 254 nm in buffer in the presence of 10^{-2} KCN. After irradiation the cyanide was removed by centrifugation and the cells suspended in warm tryptone broth + 2 μg/ml. thymidine at a concentration of 2×10^7 per ml., and shaken at 37° C. At intervals, samples were centrifuged and 1 ml. of ice-cold 5 per cent TCA was added to the cell pellet. The acid-precipitable fraction was separated by centrifugation and assayed for dimer content as previously described[1,7].

Experiment	W3110	**P3478**
1	□	■
2	○	●
3	△	▲

of P3478 has the effect of keeping the gaps open for longer times than in W3110, there was probably some repair of breaks even in the mutant strain.

We conclude that an exonuclease activity possessed by P3478, which leads to the degradation of UV-irradiated DNA, masks the excision of dimers, and interferes with the final step in excision-repair, that of restoring the integrity of the phosphodiester backbone of the DNA duplex. The identity of the exonuclease remains obscure. The possibility that there is a mutation in a nuclease other than the exonuclease activity of DNA polymerase seems unlikely at first sight, for extracts of P3478 cells do not inhibit DNA synthesis mediated by extracts of W3110 (ref. 5). The important activity in the excision model based on DNA polymerase is that of the 5′→3′ directed exonuclease[4], which can occur, *in vitro*, in the absence of polymerization[9]. Our results do not contradict this model.

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DNA Synthesis *in vitro*

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In a new *in vitro* system, DNA is synthesized semiconservatively at rates of chain growth comparable with replication *in vivo*. This DNA synthesis is also observed with a strain of *E. coli*, which lacks DNA polymerase activity *in vitro*.

DNA synthesis *in vitro*, using the classic purified DNA polymerase system of Kornberg[1], differs from replication *in vivo* in several respects. The two most important are that the *in vivo* rate is two orders of magnitude higher than the *in vitro* rate and that no net semiconservative synthesis is achieved *in vitro* with double stranded DNA as template[2]. Furthermore, it now seems likely that DNA replication is mediated by a number of different proteins which may be assembled in a replication complex at the cell membrane. The search for such a complex in its functional state *in vitro*[3-6] is, however, usually complicated by the presence of DNA polymerase, which effectively uses damaged DNA as a substrate for repair synthesis. In this and in the following article[7] we describe two new systems for DNA synthesis *in vitro* which reduce the activity of this unwanted background reaction.

Table 1. CHARACTERISTICS OF THE INCORPORATION SYSTEM

System	dCMP incorporated into acid-insoluble nucleotides during 1 min at 37° C (pmoles/10^8 cells)
Complete	14·5
Minus dTTP	0·4
Minus dATP	0·5
Minus dTTP, dATP	0·3
Minus dTTP, plus dBUTP	14·1
Minus rATP	6·2
Preincubation without 3 dNTPs for 3 min, at 37° C	11·0
Plus pancreatic deoxyribonuclease (50 μg), venom phosphodiesterase (50 units)	
3 min before and during incorporation	0·7
After incorporation	1·2
Minus 0·06 M KCl, plus 0·032 M KPO_4 (*p*H 7·5)	10·1
Minus 0·06 M KCl, plus 0·016 M KP_2O_7 (*p*H 7·5)	1·2

E. coli W 3110 *thy⁻ pol A_1* cells, prelabelled with ^{14}C-thymine (about 300 c.p.m./10^8 cells) were embedded in 2 per cent agar (2–6 × 10^9 cells/ml.). The agar was fragmented; fragments were washed and incubated with aeration at 37° C in a spheroplast forming medium (66 ml./g agar) which consists of 'Penassay' broth containing penicillin (500 units/ml.), 0·5 M sucrose, and 0·01 M $MgCl_2$. After 2 h the agar was collected by filtration and washed five times with 10 ml. of ice cold lysis buffer (0·05 M Tris-HCl, *p*H 7·5, 0·005 M $MgCl_2$, 10^{-6} M tRNA, 5 per cent 'Brij 58'). Agar samples of about 0·05 g were assayed for nucleotide incorporation in 0·2 ml. of a reaction mixture containing 0·05 M Tris, *p*H 7·5, 0·005 M $MgCl_2$, 0·08 M KCl, 10^{-4} M dithiothreitol, 10^{-3} M ATP, 2×10^{-5} M dTTP, dATP, dGTP, 2×10^{-5} M ^{3}H- or α-^{32}P-labelled dCTP (10^3 c.p.m./pmole), and 10^{-4} M deoxycytidine. After 1 min at 37° C the reaction was terminated by addition of 0·1 M EDTA (0·2 ml.). 1 M NaOH (0·5 ml.) was added and the agar was dissolved by heating to 100° C for 5 min. The acid-precipitable radioactivity was determined. The results were corrected for blank values (EDTA added at 0 min) which ranged from 0·5 to 1 per cent of the incorporation of the complete system. ^{14}C-thymine prelabel was used to normalize the incorporation values with respect to the number of cells (except for the nuclease treated samples).

We have tried to develop an *in vitro* system from *Escherichia coli* which maintains the structural integrity of DNA and membrane as far as possible, and which allows removal of the bulk of soluble components, including DNA polymerase. *E. coli* cells are embedded in a matrix of agar, the agar is fragmented, the cells are transformed into spheroplasts within the agar fragments and lysed carefully by osmotic shock. After extensive washing the agar still contains DNA and membranes. On incubation with deoxyribonucleoside triphosphates, DNA is synthesized within the agar fragments in a semiconservative manner. This enzymatic activity differs distinctly from that of DNA polymerase in several respects, and particularly in its rate of chain elongation. The *E. coli* mutant described by de Lucia and Cairns, W3110 *thy⁻ pol A_1*, which lacks DNA polymerase activity *in vitro*[8], was extremely useful in further studies of this system. Only the repair replication was reduced, whereas the other activity remained unaltered. This supports the conclusion that our system characterizes a new DNA-synthesizing activity.

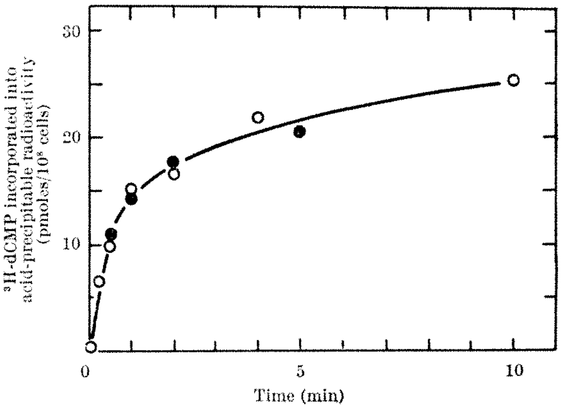

Fig. 1. Kinetics of DNA synthesis. ●, DNA synthesis in the complete system; ○, in the complete system with dBUTP instead of dTTP.

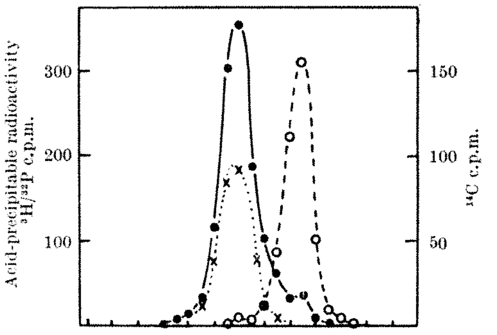

Fig. 2. Density analysis of bromouracil-labelled DNA. DNA was synthesized in 0·5 g agar incubated in 0·75 ml. of the complete reaction mixture with dBUTP replacing dTTP. After 60 s at 37° C, 0·5 ml. of a frozen solution of 0·06 M EDTA and 6 per cent 'Sarkosyl' was added and the agar was dissolved by addition of KI (1·2 g) and heating to 42° C for 15 min. The solution was diluted to 4 ml. and briefly sonicated. KI was removed by dialysis against 0·05 M Tris (*p*H 7·5)–10^{-4} M EDTA. The precipitated agar was removed by centrifugation. 2·8 g of CsCl was added to 2 ml. of the supernatant. The solution was centrifuged for 48 h at 37,000 r.p.m. in the Spinco 'SW 50' rotor. Acid-precipitable radioactivity was determined in fractions after a 4 min incubation at 100° C with 1 M NaOH (0·5 ml.). ○ - - - ○, ^{14}C prelabelled DNA; ●—●, ^{3}H DNA synthesized *in vitro*; × · · · ×, ^{32}P hybrid (bromouracil/thymine) DNA reference.

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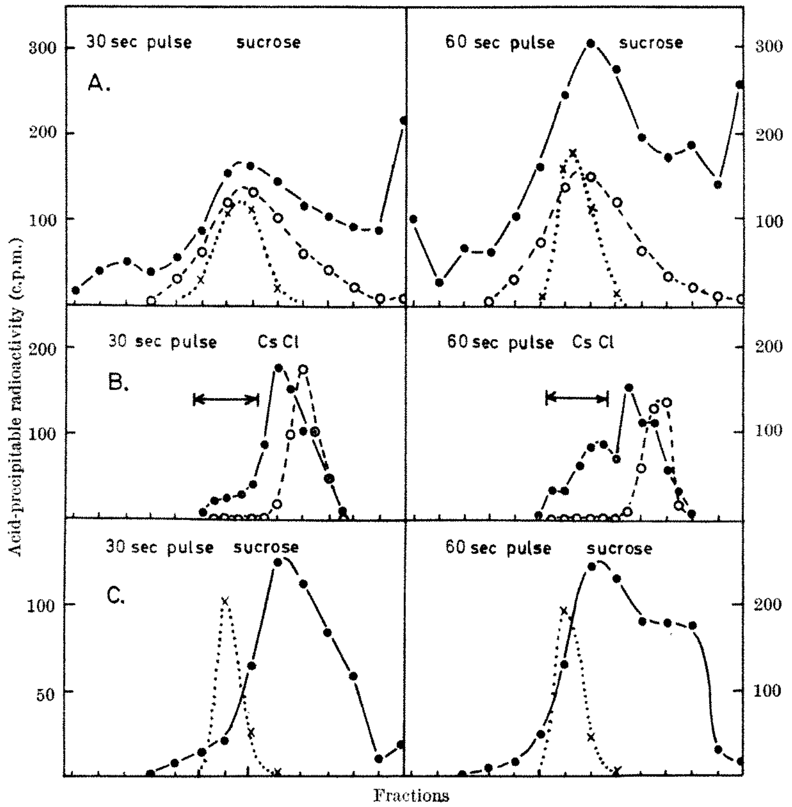

Fig. 3. Size determination. DNA was synthesized in the presence of dBUTP for 30 and 60 s. Except that sonication was replaced by mild shear the procedure was essentially as described in the legend of Fig. 2. *A*, Sedimentation analysis of a fraction of the sheared DNA. A sucrose gradient of 20 to 5 per cent sucrose in 0·01 M Tris and 2 M KI was layered on top of 2 ml. saturated CsCl. All solutions contained low molecular weight DNA (100 μg/ml.) and 'Sarkosyl' (0·1 per cent). Sedimentation was carried out in the Spinco 'SW 41' rotor for 13 h at 20° C and 30,000 r.p.m. The direction of sedimentation is from right to left. *B*, CsCl density gradient. The sheared DNA was separated from the agar by centrifugation into a cushion of CsCl. The CsCl concentration of the DNA containing fraction was adjusted to 57 per cent. After centrifugation in the 'SW 39' rotor at 30,000 r.p.m., 20° C for 48 h, fractions were collected and acid-insoluble radioactivity was determined in a sample of each fraction. The fractions containing hybrid DNA were pooled as indicated by the arrows. *C*, Sedimentation analysis of hybrid DNA. The hybrid DNA obtained from the above density gradient was dialysed against 10^{-2} M Tris (*p*H 7·5) 10^{-4} M EDTA. Sucrose gradients were prepared as described for *A* except that 0·5 M NaCl was used instead of 2 M KI. Centrifugation was carried out in the 'SW 41' rotor for 5 h at 20° and 36,000 r.p.m. ○ - - - ○, ^{14}C-prelabelled DNA; ●—●, ^{3}H-DNA synthesized *in vitro*; × ··· ×, ^{32}P-labelled fd DNA reference. Its sedimentation coefficient is $s_{20,\,w} = 31$S, determined in our conditions in an analytical ultracentrifuge.

Synthesis of DNA

Experimental details of the system are described in the legend to Table 1. The system shows properties expected for template dependent DNA synthesis *in vitro* (Table 1). Synthesis requires all four deoxyribonucleoside triphosphates and the product is sensitive to degradation by deoxyribonuclease. α-^{32}P-dCTP is incorporated just as well as ^{3}H-dCTP. Pyrophosphate ions inhibit the incorporation while phosphate ions have little influence. This suggests that deoxyribonucleoside triphosphates are the precursors for the DNA synthesized. The system is accessible to deoxyribonuclease during incubation. This finding, and the incorporation of α-^{32}P-labelled nucleotides, indicate that the incorporation takes place *in vitro* rather than in any non-disrupted cells.

Mg^{2+} is essential for the activity of the system. Excess EDTA inhibits the system reversibly, whereas the same amount of ethylene glycol-*bis*-(2-aminoethyl ether)N,N'-tetraacetic acid (EGTA) has no influence. The dependence of the described enzymatic activity on *p*H, ionic strength, Mg^{2+} concentration, temperature and especially on ATP is quite different from that of the activity of purified DNA polymerase. These differences do not prove, however, that the synthesizing enzyme is different from DNA polymerase. They might rather reflect properties of other enzymes present in the crude system and which are indirectly involved. The optimal conditions for the system are given in the legend to Table 1 and closely resemble intracellular conditions.

When repair replication by DNA polymerase is avoided by using the polymerase-negative mutant, the agar procedure is unnecessary, for DNA synthesis is observed even in ordinary lysates of spheroplasts. Shear by vortex-mixing has only little influence, whereas extensive breakage by sonication reduced this synthesis by at least a factor of ten.

DNA synthesis levels off after 1 to 2 min at 37° C (Fig. 1). The reason for this is not yet known. It seems unlikely that the short lifetime of the system is the result of exhaustion of precursors or heat inactivation. Repeated addition of 5'-deoxyribonucleoside triphosphates during incubation and preincubation of the system at 37° C without deoxyribonucleoside triphosphates has little influence on the final amount of DNA synthesized. The initial rate of synthesis calculated from incorporation after 30 s corresponds to the incorporation of 5×10^5 nucleotides per min per spheroplast.

Characteristics of DNA Synthesis

To demonstrate semi-conservative synthesis, bromodeoxyuridine triphosphate (dBUTP) was used as a density label: dTTP can be replaced by dBUTP without a reduction in DNA synthesis (Table 1 and Fig. 1). This is to be expected, for *in vivo* dBUTP and dTTP seem to be equally suitable for DNA polymerization (unpublished results of H. E. S. and Voss). The DNA synthesized *in vitro* in the presence of dBUTP instead of dTTP was broken by sonication and analysed in CsCl density gradients. The newly synthesized material had the hybrid density expected of semiconservatively synthesized DNA, which consists of one old light strand and one heavy strand containing the ^{3}H label (Fig. 2). On denaturation, these strands separate in a CsCl gradient and band at positions of heavy and light single strands. As Dr Herrmann has shown, the amount of the parental DNA which is transferred to hybrid density is about 0·5 per cent.

The rate of chain elongation can be calculated from the size of the pieces of newly synthesized DNA and the time required for their synthesis. To determine the size, DNA was synthesized in the presence of dBUTP for 30 or 60 s. The agar was dissolved in KI and the solution sheared. Sedimentation analysis of the sheared DNA showed a broad distribution of molecular weights (Fig. 3*A*). The average molecular weight is about 3×10^7 daltons as calculated from the sedimentation coefficient of 30S (ref. 9). To determine the size of the newly synthesized hybrid molecules the DNA was first removed from the agarose by a preparative sucrose gradient centrifugation and collected on a CsCl cushion. It was then subjected to CsCl density gradient centrifugation. The density distribution is shown in Fig. 3*B*. That fraction of the newly synthesized DNA which banded at the position of hybrid DNA was analysed for size distribution in a sucrose gradient (Fig. 3*C*). The largest molecules which had banded at hybrid density sedimented at about 30S, which corresponds to a molecular weight of 3×10^7 daltons. When the sheared DNA was denatured and the heavy single strands were isolated from a CsCl density gradient, the largest molecules found had the same sedimentation rate as denatured λ DNA (which has a molecular weight of 15×10^6 daltons). From these data the rate of chain elongation is calculated to be $1{\cdot}5 \times 10^3$ nucleotides per second—very close to the rate of DNA replication *in vivo*[10].

The size distribution of the hybrid DNA (Fig. 3*C*) does not permit an exact calculation of the average size of the newly synthesized piece of DNA, mainly because the selection of hybrid DNA is a preferential selection of small molecules. As expected, the average sedimentation velocity of ^{3}H-labelled DNA which bands at light density was greater than that of hybrid DNA.

The rate of total incorporation (5×10^5 nucleotides/min/spheroplast) is about five times higher than the estimated rate of chain elongation (10^5 nucleotides/min). If all the DNA were synthesized at a rate of 10^5 nucleotides/min, the rate of total synthesis could be explained by synthesis at two to three growth points. This number is not unreasonable. There may well be more growth points per spheroplast, but, on the other hand, not all of them might be active in the *in vitro* system.

Relationship with Replication *in vivo*

Our *in vitro* system can synthesize DNA at rates comparable with replication *in vivo*. The total amount synthesized *in vitro* within 1 min is of the same order of magnitude as the amount of DNA synthesized *in vivo* by replication within the same time. Although DNA synthesis continues for only about 1 min, the short lifetime of the system seems less disturbing because the size of the DNA which is copied within this time is very large and corresponds to 10^5 base pairs. It has not yet been shown that both DNA strands are synthesized in this system or that DNA synthesis really occurs on double stranded DNA. We believe, however, that we are observing a limited continuation of real replication at the original growth point.

This system is still very crude. Preliminary experiments indicate that it may be difficult to purify the synthesizing apparatus, free it from its original DNA and use it for synthesis on external template DNA. As demonstrated by Knippers and Strätling[7], a possible first step in purification is the isolation of membrane fractions which display a similar DNA synthesizing activity.

Because DNA synthesis is also observed in sheared bacterial lysates neither integrity of the bacterial chromosome nor an active swivel mechanism of the kind postulated for replication[11] can be necessary for this kind of DNA synthesis. That DNA synthesis is observed *in vitro* when a DNA polymerase-negative mutant is used does not exclude the possibility that the synthesizing enzyme is DNA polymerase associated with other components in a special complex. The more likely interpretation, however, is that our system makes use of another distinct replicating activity, responsible for replication *in vivo*.

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The DNA Replicating Capacity of Isolated *E. coli* Cell Wall–Membrane Complexes

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The only DNA synthesizing activity left in a bacterial mutant lacking DNA polymerase is associated with the cell membrane.

THE DNA polymerase described by Kornberg[1,2] has afforded many clues as to the nature of DNA polymerization, but it is not certain whether it constitutes part of the cellular machinery for the replication of genetic material *in vivo*. Moreover, many of its properties[3] can be explained in terms of a DNA repair enzyme. Attempts to characterize the process of DNA replication in a cell free system by measuring the incorporation of radioactive deoxyribonucleoside triphosphates into acid-precipitable DNA[4,5] have been obscured by the high activity of the DNA polymerase. Some clarification of these difficulties has been obtained, however, by the use of the mutant of *Escherichia coli* described by de Lucia and Cairns, which possesses almost no DNA polymerase activity but nevertheless grows like wild type strains in the usual conditions[6].

We have used the DNA of bacteriophage φX 174 to determine the DNA replicating potential of cell extracts and of cell-wall membrane complexes prepared from a derivative of the DNA polymerase mutant. The various intracellular forms of φX DNA are small enough (mol. wt. $3{\cdot}4 \times 10^6$) to be handled without much danger of breakage. *E. coli* DNA (mol. wt. about 3×10^9), on the other hand, is broken into many pieces during conventional extraction procedures.

Early in the reproduction cycle of phage φX 174, the parental circular double stranded DNA molecule (replica-

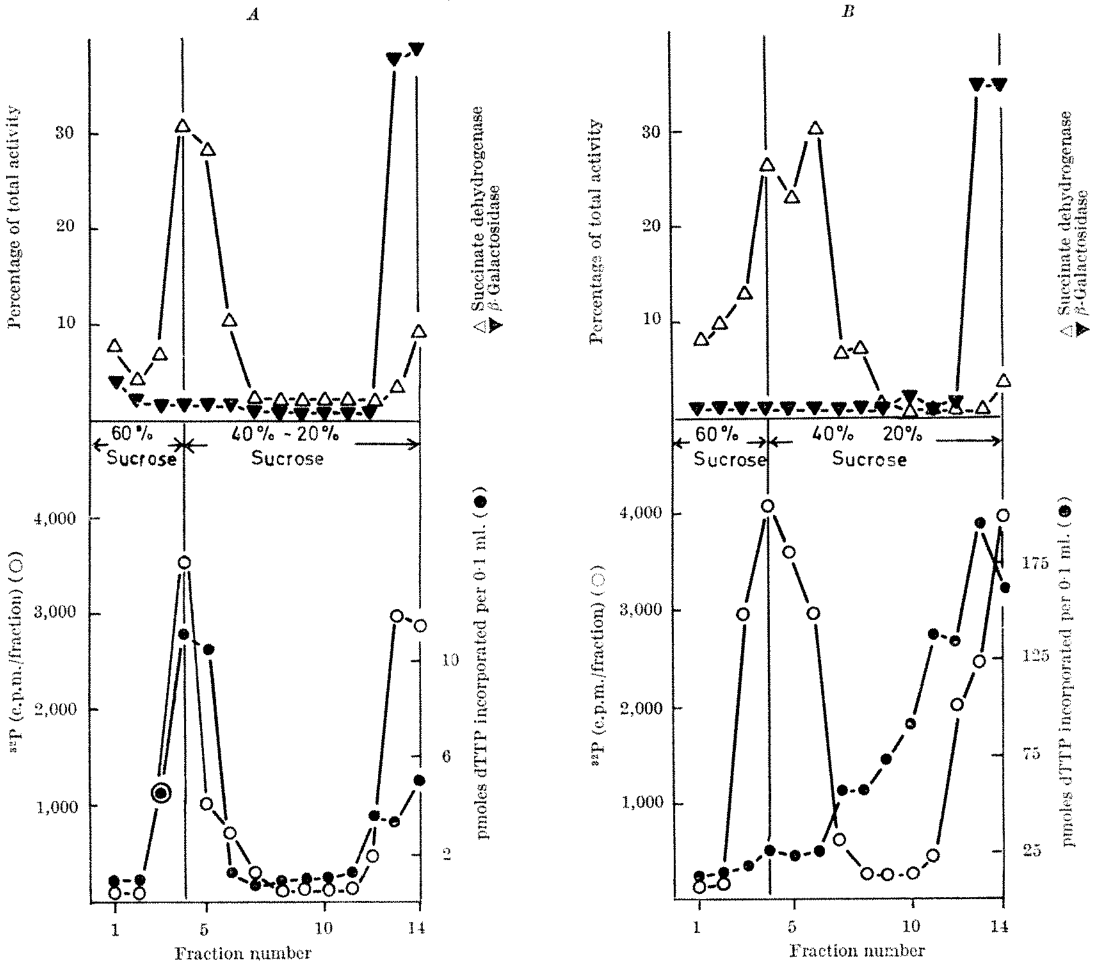

Fig. 1. Sucrose gradient analysis of cell extracts. ^{32}P-labelled φX-infected cells (multiplicity about 6) were lysed as described in the text. A 3 ml. sample of the lysate was then placed on a sucrose gradient. A linear 40 to 20 per cent sucrose gradient was formed above an underlayer of 7 ml. of 60 per cent sucrose. The sucrose solutions were buffered by 0·01 M MOPS (*p*H 7·2)–0·1 M KCl–0·005 M $MgSO_4$–0·0025 M mercaptoethanol. Centrifugation time was 45 min at 25,000 r.p.m. and 2° C in the 'SW 27' rotor of the Spinco centrifuge. Sedimentation is from the right to the left. In aliquots of each fraction the activities of the enzymes succinate dehydrogenase[14] and β-galactosidase (with *p*-nitrophenol-β-galactoside) were tested. The protein concentration was determined by the Lowry reaction[15] (not shown). About 25 per cent of the total cellular protein sedimented with the membrane-wall complex; the remainder was found in the two or three top fractions of the sucrose gradient. A 0·1 ml. sample of each fraction was then mixed with 0·1 ml. MOPS buffer: 0·05 M, *p*H 7·2 containing 5 nmole each of dATP, dGTP. dCTP and TTP plus 0·5 μCi ^{3}H-TTP (9·8 Ci/mmole), 50 nmole ATP and 5 μg "activated" calf thymus DNA. Incubation was for 20 min at 30° C. *A*, Analysis of an extract of about 2×10^{10} ^{32}P-φX-infected *E. coli* H 560 cells; *B*, extract of about $4 \cdot 5 \times 10^{10}$ ^{32}P-φX-infected *E. coli* C cells.

tive form, RF-DNA) replicates semiconservatively to produce circular double stranded daughter DNA molecules (reviewed in ref. 7). We believe that this production of double stranded DNA rings is an example of the ring-to-ring replication, which frequently occurs in microorganisms. We bore in mind, of course, that φX 174 RF-DNA replicates semiconservatively only (1) when one of its composing DNA strands is nicked[8] and an active product of the cistron VI of the phage genome is present[9]; (2) when the replicating DNA is associated with the cell membrane[10]; and (3) when the cellular "replicating apparatus" operates normally[7]. All these conditions are observed by extracting φX 174 infected bacteria gently enough to leave the cellular membrane functionally intact.

Preparation of DNA-membrane Complexes

Exponentially growing *E. coli* H 560 cells (a φX-sensitive derivative of the DNA polymerase mutant of de Lucia and Cairns, kindly supplied by Dr Hoffmann-Berling) were infected with ^{32}P-labelled φX phages in the presence of 30 μg/ml. chloramphenicol. About 20 min later the infected cells were quickly cooled in a methanol solid CO_2 bath, pelleted and washed with a 20 per cent sucrose solution buffered by 0·05 M morpholinopropane sulphonic acid (MOPS)[11] and 0·005 M ethylene glycol-*bis*(2-aminoethyl ether)-N,N′-tetraacetic acid (EGTA)[12] at *p*H 7·0. Between 1×10^{10} and 3×10^{10} cells were then resuspended in 1 ml. of the MOPS–EGTA buffer and treated for 30 min with 50 μg lysozyme/ml. in an ice bath, 0·5 per cent 'Brij 58' (ref. 13) and 0·005 M $MgSO_4$ were then added. After 2–5 min at room temperature the lysate was centrifuged for 45 min through a sucrose gradient (Fig. 1*a*). More than 85 per cent of the *E. coli* DNA and about 50 per cent of the parental ^{32}P-φX-DNA were recovered together with the cell-wall membrane complex as a viscous turbid band floating on top of the 60 per cent sucrose at the bottom of the centrifuge tube. Non-lysed cells sedimented through the shelf. The cell-wall membrane complex was identified by the activity of the enzyme succinate dehydrogenase[14] bound to this structure and by testing for protein[15]. The sucrose gradient fractions containing most of the cytoplasmic material were identified by the presence of the enzyme β-galactosidase (after induction with 5×10^{-4} M IPTG before infection with φX).

Without added primer DNA, radioactive deoxynucleoside triphosphates were incorporated into acid precipitable

DNA preferentially in the "membrane" fractions; after addition of "activated" calf thymus DNA[1] as primer, the polymerizing activity in the membrane fractions did not increase, while a small peak of polymerizing activity appeared in the cytoplasmic fraction of the gradient—probably corresponding to the basic level of DNA polymerase-like activity observed by de Lucia and Cairns[6] in their mutant. Fig. 1*b* illustrates a similar experiment with the common φX host strain, *E. coli* C. After addition of activated calf thymus DNA to the fractions, a much larger DNA polymerizing activity can be detected throughout the sucrose gradient. Its biochemical properties (*p*H optimum, magnesium dependence, insensitivity towards *p*-chloromercuribenzoate and so on) support the conclusion that the incorporating activity in extracts of *E. coli* C which is not associated with the membrane corresponds largely to Kornberg's DNA polymerase. Results similar to those of Fig. 1*b* have been obtained with extracts of a H 560 revertant possessing normal DNA polymerase levels.

Membrane-associated DNA Synthesis

In the experiment of Fig. 2, the DNA polymerizing activity of the wall-membrane complex was followed for a 30 min incubation period at 30° C. Incorporation of ^{3}H-TTP into acid precipitable material started soon after placing the sample at 30° C and continued for 5–10 min. During this time, between 1×10^4 and 3×10^4 thymidine phosphate molecules per cell were polymerized. We do not know why incorporation stops after a few minutes at 30° C. When partially purified DNA polymerase was added to the membrane preparation it was active for at least 30 min, demonstrating that the limited lifetime of the membrane associated polymerizing activity is probably not because the deoxynucleoside triphosphates are exhausted. No conditions have been found to prolong the polymerization time of the membrane system (at a given temperature), and no stimulatory effect by a cytoplasmic component could be observed. A short ultrasonic treatment of the membrane destroyed its synthesizing activity, even if the system was subsequently supplemented with intact φX RF-DNA or highly polymerized *E. coli* DNA. Table 1 summarizes other properties of the membrane system.

Table 1. PROPERTIES OF THE MEMBRANE SYSTEM

	dTTP, incorporated into acid precipitable material (pmoles/10^9 cells)
Complete system*	63
– dATP, – dGTP, – dCTP	< 6
– ATP	40
– Mg^{2+}	< 6
– KCl	23
– Mercaptoethanol	58
+ Pancreatic deoxyribonuclease (15 μg/ml.)	< 6
+ Pancreatic ribonuclease (25 μg/ml.)	62
+ Pronase (10 μg/ml.)	< 6
+ 'Sarkosyl' (0·05 per cent)	< 6
+ 'Brij 58' (0·5 per cent)	63
+ *p*-Chloromercuribenzoate (3×10^{-4} M) mercaptoethanol	8

*0·3 ml. membrane-DNA complexes (corresponding to $2–3 \times 10^9$ cells, see Fig. 1) in 0·01 M MOPS (*p*H 7·2), 0·08 M KCl, 0·005 M $MgSO_4$, 0·0025 M mercaptoethanol were incubated for 30 min at 30° C with 16 nmole each of dGTP, dATP, dCTP and TTP, 0·5 μmole ATP and 1·2 μCi ^{3}H-TTP (9·3 mCi/mole).

At the end of the incubation period (usually after 20 min at 30° C) EDTA was added (final concentration, 0·01 M), and the DNA was extracted with 'Sarkosyl' (Geigy, 'NL-90'; final concentration, 0·5 per cent) and pronase (100 μg/ml.) for 2–4 h at room temperature. After dialysis the DNA was purified by sucrose gradient centrifugation. Between 80 and 90 per cent of the incorporated radioactivity appeared in *E. coli* DNA, and only 10–20 per cent in phage φX-DNA, according to DNA–DNA annealing techniques[16,17]. (A ratio of incorporated c.p.m. (^{3}H) in *E. coli* DNA to those in φX-DNA of about four has been determined, which applies too for chloramphenicol treated φX infected H 560 cells after a 1 min pulse at 25° C with ^{3}H-thymidine *in vivo*: unpublished results of R. K.) Fig. 3 demonstrates that the parental ^{32}P-labelled φX DNA largely remains intact during the preparation and during the incubation period. More than 80 per cent of the parental φX DNA sedimented in two peaks corresponding to the supercoiled form of double stranded φX DNA (RF-I) and to the extended ring form (RF-II), respectively. The remaining ^{32}P label was found as a component of unknown structure floating on top of the CsCl shelf at the bottom of the centrifuge tube, which prevents large DNA pieces from pelleting. When the host cell DNA was prelabelled with ^{14}C-thymine most of *E. coli* DNA was also detected above the CsCl shelf. The ^{3}H-labelled incorporation products appeared predominantly at three places on the sucrose gradient (Fig. 3): (i) on top of the CsCl shelf; this fast sedimenting DNA does not hybridize with denatured φX RF (refs. 16 and 17) and consists most probably of host cell DNA; (ii) a broad peak of DNA pieces sedimenting with rates of about 10S; we find these DNA pieces in experiments with membranes of infected as well as of uninfected cells; (iii) a peak of ^{3}H-labelled DNA detectable only in φX infected cells sedimenting at 16S, frequently with leading shoulders up to 20S. This peak of ^{3}H-labelled DNA hybridizes with φX viral DNA and with an equal amount of denatured φX RF (1·5 to 1·7 times more efficient), indicating that these products consist of both complementary polynucleotide strands of the φX RF.

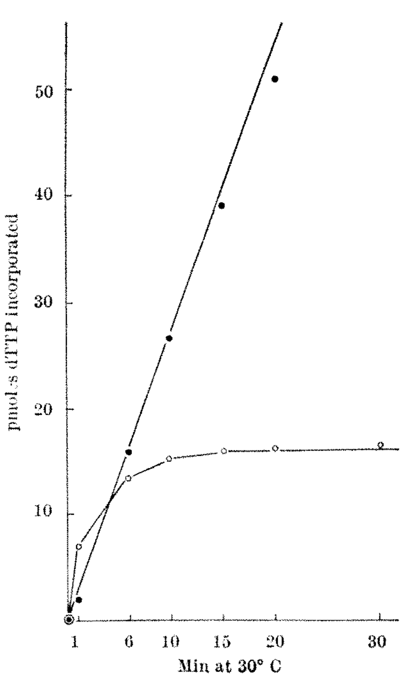

Fig. 2. Kinetics of incorporation of radioactive deoxynucleoside triphosphates into DNA. Preparation of the membrane fractions of ^{32}P-φX-infected *E. coli* H 560 cells is described in the text and in the legend to Fig. 1. To a 2 ml. sample of the membrane preparation in cold 0·1 M KCl–0·005 M $MgSO_4$–0·0025 M mercaptoethanol–0·05 M MOPS buffer deoxynucleoside triphosphates and ATP were added in the same final concentrations and specific radioactivity as in the legend to Fig. 1. Samples of 0·15 ml. were removed at the indicated times after placing the reaction mixture into a 30° C water bath. The ^{32}P radioactivity of a given number of cells was determined before lysis and served as reference. To another 2 ml. sample of the same membrane preparation 0·2 ml. of 5×10^{-3} M *p*-chloromercuribenzoate was added (see Table 1). After 10 min at 0° C, deoxynucleotide triphosphates and ATP were added (see Fig. 1) together with 20 μg/ml. "activated calf thymus" DNA and about 1 unit of DNA polymerase[3]. Incorporation of ^{3}H-TTP into acid precipitable material was linear for 30 min. The *p*H of the reaction mixture was in both experiments adjusted to *p*H 7·2, the optimum for the membrane bound activity. The *p*H optimum for partially purified DNA polymerase in the same experimental conditions is around *p*H 8·1. ○—○, "Membrane system"; ●—●, DNA polymerase.

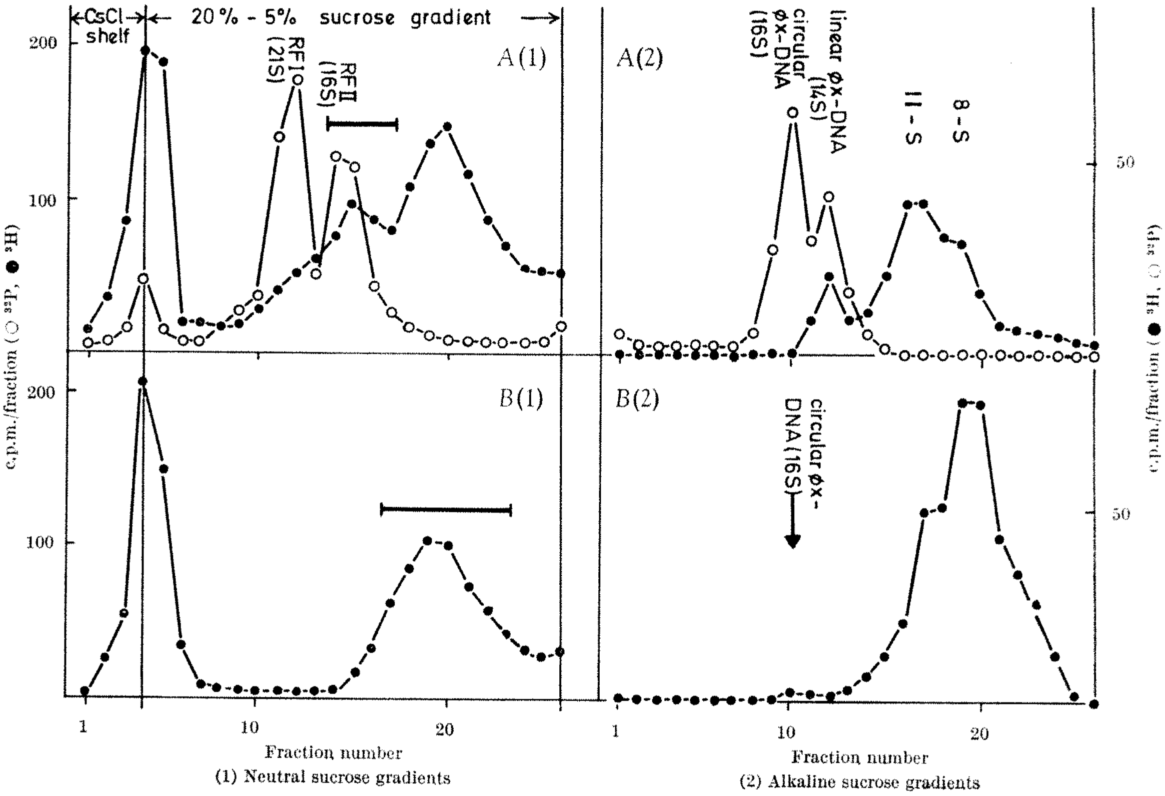

Fig. 3. Sucrose gradient analysis of *in vitro* synthesized DNA. *A*, DNA produced in cell free extracts of ^{32}P-φX-infected cells. (1) The 'Sarkosyl'-pronase extracted DNA was sedimented for 13 h at 4° C at 24,000 r.p.m. in the Spinco 'SW 27' rotor through a 20 to 5 per cent sucrose gradient formed on a 5 ml. CsCl shelf (1·2 g CsCl/ml, 20 per cent sucrose). The sucrose was dissolved in 0·3 M NaCl–0·05 M Tris-HCl–0·005 M EDTA (*p*H 8). (2) Part of fractions 14–17 of the neutral sucrose gradients was recovered, dialysed and denatured with alkali. The denatured DNA was sedimented for 22 h at 27,000 r.p.m. through a 20 to 5 per cent sucrose gradient in 0·01 M EDTA–0·3 M NaOH, *p*H 12·5. *B*, DNA produced in cell free extracts from uninfected cells. (1) Centrifugation conditions: as given. (2) Part of fractions 17–23 of the neutral gradient was recovered and after dialysis denatured with alkali. Alkaline sucrose gradient centrifugation: as above. ^{32}P-labelled φX DNA, extracted from phage particles, served as sedimentation marker. The peak of the 16 S circular φX DNA is marked by the arrow. Sedimentation is from the right to the left.

Phage DNA

A series of experiments in which thymidine triphosphate was replaced by 5-bromodeoxyuridine triphosphate (BUTP, a gift of Dr H. Schaller) and ^{3}H-TTP by ^{3}H-dCTP, respectively, showed that the ^{3}H-labelled φX DNA is the product of an ordered replication process. The φX-specific DNA corresponding to the fractions 14–17 of Fig. 3*A* was recovered from the sucrose gradient and centrifuged to equilibrium in a CsCl gradient (Fig. 4). Three buoyant density classes of DNA can be seen at the end of the equilibrium run: (i) most (80–90 per cent) of the parental ^{32}P-DNA and a small fraction of the ^{3}H-labelled material had the density of a double stranded phage DNA (1·72 g/ml.) of which both strands contain thymine (light–light); (ii) less than 10 per cent of the parental ^{32}P-DNA and 60–70 per cent of the ^{3}H-labelled DNA banded at the buoyant density of a hybrid DNA, composed of one heavy bromouracil and one light thymine containing strand; (iii) roughly 20 per cent of the ^{3}H-labelled incorporation products exhibited the high buoyant density, expected for a double stranded DNA piece of which both strands contain bromouracil instead of thymine (heavy–heavy DNA).

Thus less than one parental RF molecule per cell participates in the semiconservative replication of the membrane system, and replication of φX DNA most often stops after one round of replication. Additional rounds of φX RF replication are initiated only infrequently. In the experiment of Fig. 4, the infectivity of the φX DNA molecules was tested with cells converted to spheroplasts according to Guthrie and Sinsheimer[18]. Most of the infective DNA molecules appear at the fully light position (these molecules were formed in the cell before the lysis); a shoulder of this main peak of infectivity extends into the band of hybrid DNA; and a small but clearly visible infectivity peak corresponds to the fully heavy ^{3}H-labelled DNA. It therefore seems that a small number of intact φX DNA molecules with the complete genetic information of the phage is formed in the membrane system. This result suggests that many of the components necessary for double stranded phage DNA replication are associated with the cell membrane. A possible exception might be a ring closing activity, such as that of polynucleotide ligase, because the largest visible φX specific DNA strand labelled in the membrane system sediments at 14S like a linear φX DNA of one genome length (see Fig. 3*A* (2)). Addition of NAD (10^{-3} M), the cofactor of the polynucleotide ligase[20], with or without cytoplasmic proteins did not change this result. The bromouracil-labelled φX RF-DNAs which are infective are presumably composed of linear polynucleotide strands forming a double stranded DNA circle by means of overlapping complementary single stranded ends.

E. coli DNA

Part of the ^{3}H-labelled *E. coli* DNA (varying in different experiments from 40 to 80 per cent of the total radioactive bacterial DNA) sedimented with most of the parental bacterial DNA and was found at the end of the centrifuge run on top of the CsCl shelf. The remaining ^{3}H-labelled *E. coli* DNA had a sedimentation rate of about 10S

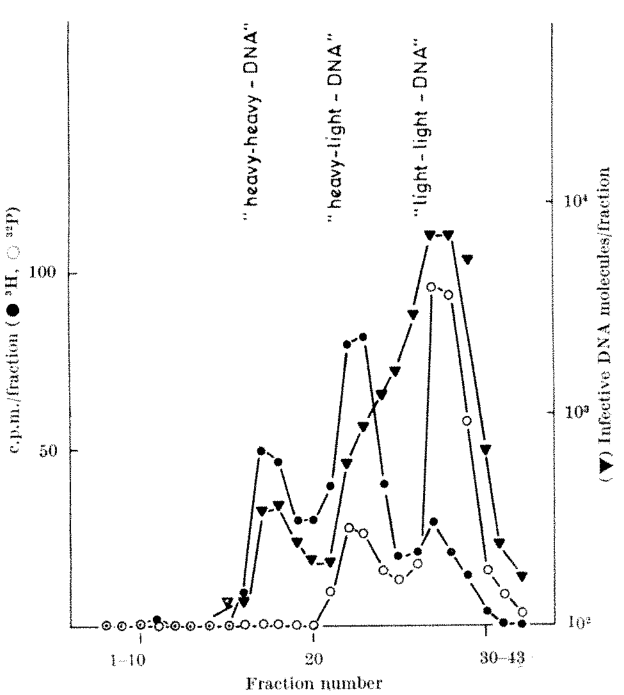

Fig. 4. CsCl equilibrium density centrifugation. A cell free extract of ^{32}P-φX-infected cells was incubated in the conditions described in Table 1, except that TTP was replaced by BUTP and ^{3}H-TTP by ^{3}H-dCTP. The part of the sucrose gradient corresponding to the fractions 14–17 of Fig. 3*A* was recovered, brought to a density of 1·73 g/ml. with CsCl and centrifuged for 55 h at 34,000 r.p.m. in the Spinco 'SW 65' rotor (volume 3·6 ml.). Forty-three fractions were collected, each into 1 ml. sterile Tris-EDTA buffer; 0·05 ml. samples were removed, diluted 1 : 40 into Tris-EDTA buffer and investigated in the spheroplast assay of Guthrie and Sinsheimer[18]. Results are given in relative units: infective DNA molecules/ml. The remainders of the samples were precipitated with 10 per cent trichloroacetic acid on 'Millipore' filters and counted.

(Fig. 3*B* (1)). On alkaline denaturation the 10*S* DNA decomposed into DNA fragments sedimenting at 4·5S, 8S and 11S, respectively. The various fragments were usually identified only as shoulders of the 8S component (Fig. 3*B* (2)). Similar distributions of DNA fragments have been observed in the presence and absence of NAD (10^{-3} M), with or without cytoplasmic proteins. The 10S *E. coli* DNA is double stranded as judged from its buoyant density in CsCl gradients and bands at a density of "hybrid" (bromouracil-thymine) DNA when synthesized with BUTP instead of TTP. It is not yet known whether the 10S pieces in our system are precursors of that fraction of the ^{3}H-labelled *E. coli* DNA which sediments with the macromolecular DNA.

Preliminary estimates for the initial rate of chain elongation in the membrane system are similar to the synthesis rates observed in the agar embedded cells of Smith, Schaller and Bonhoeffer[20]; that is, polymerization of nucleotides proceeds close to the chain growth rates in living cells, although for a very short time period.

Our results demonstrate that much of the DNA replicating machinery is indeed associated with the cell membrane; and that there are at least two functionally different DNA polymerizing activities in the bacterial cell: the "classical" DNA polymerase and a membrane bound "DNA replicase", which may or may not contain the DNA polymerase as an active component. The two DNA synthesizing activities can be distinguished by several criteria. The most striking properties of the membrane system are the very short active period of DNA synthesis, its sensitivity towards *p*-chloromercuribenzoate, and its association with a large cellular structure.

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Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei

by

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Further evidence is put forward for the inducibility of chromosome condensation, particularly its consequences in interphase cells that are not ready for mitosis.

WE have shown for multinucleate HeLa cells formed by the fusion of cells from different phases of the cell cycle that DNA synthesis and mitosis are inducible[1]. Here we present further evidence for the inducibility of chromosome condensation and the consequences of such an induction in interphase (*G*1, *S*, and *G*2) cells which are not ready to enter mitosis. In heterophasic multinucleate cells formed by the fusion of *S* and *G*2 cells, the initiation of mitosis in one or more of the advanced nuclei resulted in the premature chromosome condensation (PCC) of the lagging nucleus or nuclei. Premature chromosome condensation, which is a kind of atypical mitosis without the mitotic

Table 1. SYNCHRONY IN THE FIRST MITOSIS AFTER CELL FUSION

Type of cell fusion		Binucleate		Trinucleate		Tetranucleate	
		Homophasic	Heterophasic	Homophasic	Heterophasic	Homophasic	Heterophasic
$G1/S^*$	Number of asynchronous mitoses	2^a	1^b	0	0	0	0
	Number of synchronous mitoses	332	83	112	56	25	24
	Per cent of asynchronous mitoses	0·6	1·2	0	0	0	0
$G1/G2^*$	Number of asynchronous mitoses	1^c	0	0	1^d	0	0
	Number of synchronous mitoses	402	70	119	60	25	25
	Per cent of asynchronous mitoses	0·25	0	0	1·6	0	0
$S^*/G2$	Number of asynchronous mitoses	6^e	6^f	0	10^g	0	6^h
	Number of synchronous mitoses	313	49	127	81	51	70
	Per cent of asynchronous mitoses	1·9	10·9	0	11·0	0	7·9

Details of the various types of cells grouped under each superscript are listed below. I denotes interphase; P, prophase; M, metaphase. The digits to the left of the parentheses indicate the number of such cells.

$G1/S^*$ fusion: a: $2(M^*/I^*)$
b: $1(I^*/M)$
$G1/G2^*$ fusion: c: $1(P/I)$
d: $1(2M^*/P)$
$S^*/G2$ fusion: e: $1(P^*/I^*)$; $2(I/M)$; $3(I/P)$
f: $3(I^*/M)$; $3(I^*/P)$
g: $3(2I^*/M)$; $1(I^*/P^*/P)$; $1(I^*/M^*/M)$; $2(P^*/2M)$; $1(I^*/2P)$; $2(I^*/2M)$
h: $1(2I^*/P^*/P)$; $2(2I^*/2P)$; $1(2I^*/2M)$; $1(I^*/M^*/2M)$; $1(I^*/I/2P)$

* Indicates prelabelled nucleus.

spindle, could be readily induced in interphase cells by fusing them with mitotic cells. Induction of chromosome condensation in $G1$ and $G2$ nuclei resulted in the appearance of chromosomes with single and double chromatids respectively, whereas the S nuclei yielded unevenly condensed chromatin.

We used HeLa cells grown as suspension cultures in Eagle's minimal essential medium supplemented with sodium pyruvate, glutamine and 5 per cent foetal calf serum. The culture flasks were gassed with a mixture of carbon dioxide (5 per cent) in air. We have already described[1] the procedures for obtaining synchronous populations of $G1$, S and $G2$ and the technique of cell fusion by UV-inactivated Sendai virus. Mitotic cells prelabelled with ^{3}H-thymidine (0·05 μCi/ml.; 6·7 Ci/mmole) were obtained by treating the cells with colcemide ($6{\cdot}8 \times 10^{-7}$ M) for 19 to 24 h. The method of preparation of the multinucleate cells for autoradiography and the terminology used to describe various types of multinucleate cells are also given in ref. 1. Chromosome preparations were made by giving a hypotonic treatment before the cells were centrifuged on to the slides by cytocentrifuge (Shandon-Elliott). After fixation in 3 : 1 absolute ethanol and glacial acetic acid for 5 min and hydrolysis in 1 N HCl at 60° C for 2·5 min the cells were stained with crystal violet.

Mitotic Synchrony

The incidence of asynchronous mitoses in the first division following cell fusion in multinucleate homo- and heterophasic cells resulting from $G1/S$, $G1/G2$ and $S/G2$ fusions is shown in Table 1. It is clear that in both $G1/S$ and $G1/G2$ fusions the incidence of mitotic asynchrony in the heterophasic as well as in homophasic cells was very small. In the $S/G2$ fusion, however, the incidence of mitotic asynchrony in heterophasic cells was about 10 per cent, significantly greater than that in homophasic cells. Further, the nuclei which first entered mitosis were without exception the $G2$ and not the S phase. The small incidence of mitotic asynchrony in $G1/S$ and $G1/G2$ fusions suggests that these heterophasic components can equilibrate their resources and reach mitosis together[1]. On the other hand, for at least a portion of the population of the $G2/S$ cells, the $G2$ nucleus entered mitosis while the S nucleus remained in interphase. The asynchronous entry of the $G2$ nucleus into mitosis sometimes induced similar changes in the S nucleus producing the phenomenon of premature chromosome condensation (PCC), as shown in Fig. 1. The PCC in multinucleate cells was characterized by the presence of one or more normal sets of chromosomes derived from the $G2$ nuclei along with one or more S nuclei which had undergone irregular condensation of chromatin.

The incidence of PCC was greatest in multinucleate cells containing a large proportion of $G2$ to S nuclei. The greater the ratio of $G2$ to S nuclei (3 : 1 and 2 : 1), the greater was the incidence of PCC of the S nuclei, both initially and throughout the period of investigation (Fig. 2). The greatest incidence of PCC in these cells

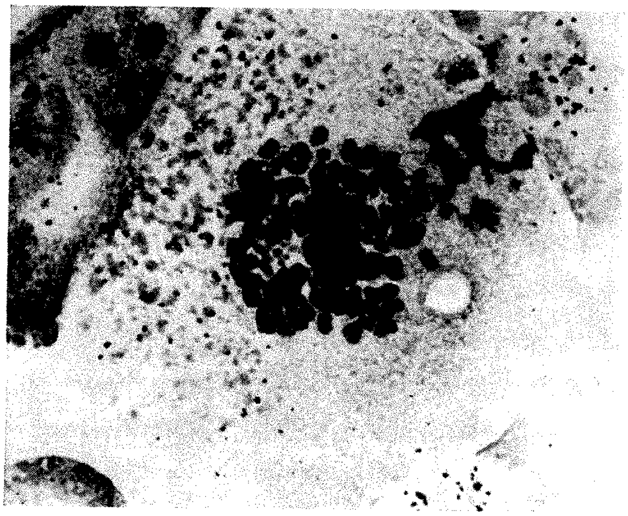

Fig. 1. Premature chromosome condensation in a heterophasic binucleate cell formed by the fusion of S and $G2$ cells. The S cells were labelled with ^{3}H-thymidine before cell fusion while the $G2$ cells were not. The initiation of mitosis in the $G2$ nucleus resulted in the premature condensation of the S-chromatin which could be identified by the silver grains in the autoradiograph.

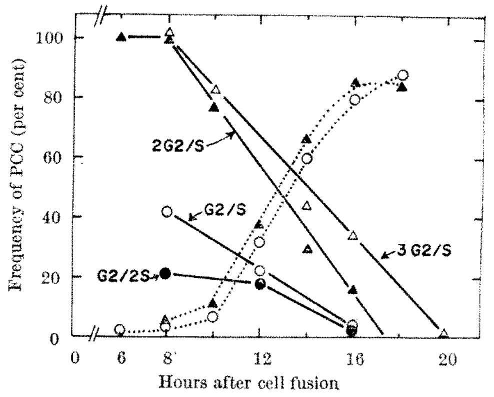

Fig. 2. The frequency of premature chromosome condensation (PCC) in various classes of heterophasic $S/G2$ cells as a function of time after the completion of cell fusion. The S nuclei were prelabelled with ^{3}H-thymidine. The mitotic accumulation functions (represented by the dotted lines) were collected from a different experiment in which colcemide was added immediately after cell fusion between S and $G2$ populations. The data on the frequency of PCC in various classes were collected from a similar experiment to which no colcemide was added following cell fusion. △—△, $3G2/1S$; ▲—▲, $2G2/1S$; ○—○, $G2/S$; ●—●, $G2/2S$. The mitotic accumulation functions for the classes are $2G2/S$ (▲ - - - ▲) and $G2/S$ (○ - - - ○).

occurred when the wave of mitoses was just beginning (see the dotted lines in Fig. 2). This suggests that the most likely multinucleate cells to contain prematurely condensed chromosomes are those that entered division as the earliest of their class. These cells presumably contained an advanced *G*2 nucleus which entered mitosis asynchronously, thus inducing PCC in the lagging "*S*" nucleus. The decrease in the frequency of PCC as a function of time (Fig. 2) indicates that those nuclei of the heterophasic cells which remain in interphase for a longer time after cell fusion have a better chance of achieving mitotic synchrony and consequently a smaller incidence of PCC.

Fusion with Synchronized Cells

The premature condensation of chromatin of the *S* nuclei after the fusion between *G*2 and *S* phase cells strongly suggested that a mitotic inducer was present in either late *G*2 or mitotic cells and that it was capable of pulling a lagging nucleus into mitosis in a dose-dependent manner. In the following experiments synchronized mitotic cells (blocked by either colcemide or N_2O) were fused with synchronous populations of *G*1, *S* and *G*2 cells to see whether mitosis could be induced in these interphase nuclei. When mitotic cells were fused with *G*1 and *G*2 cells, there was a rapid condensation of the

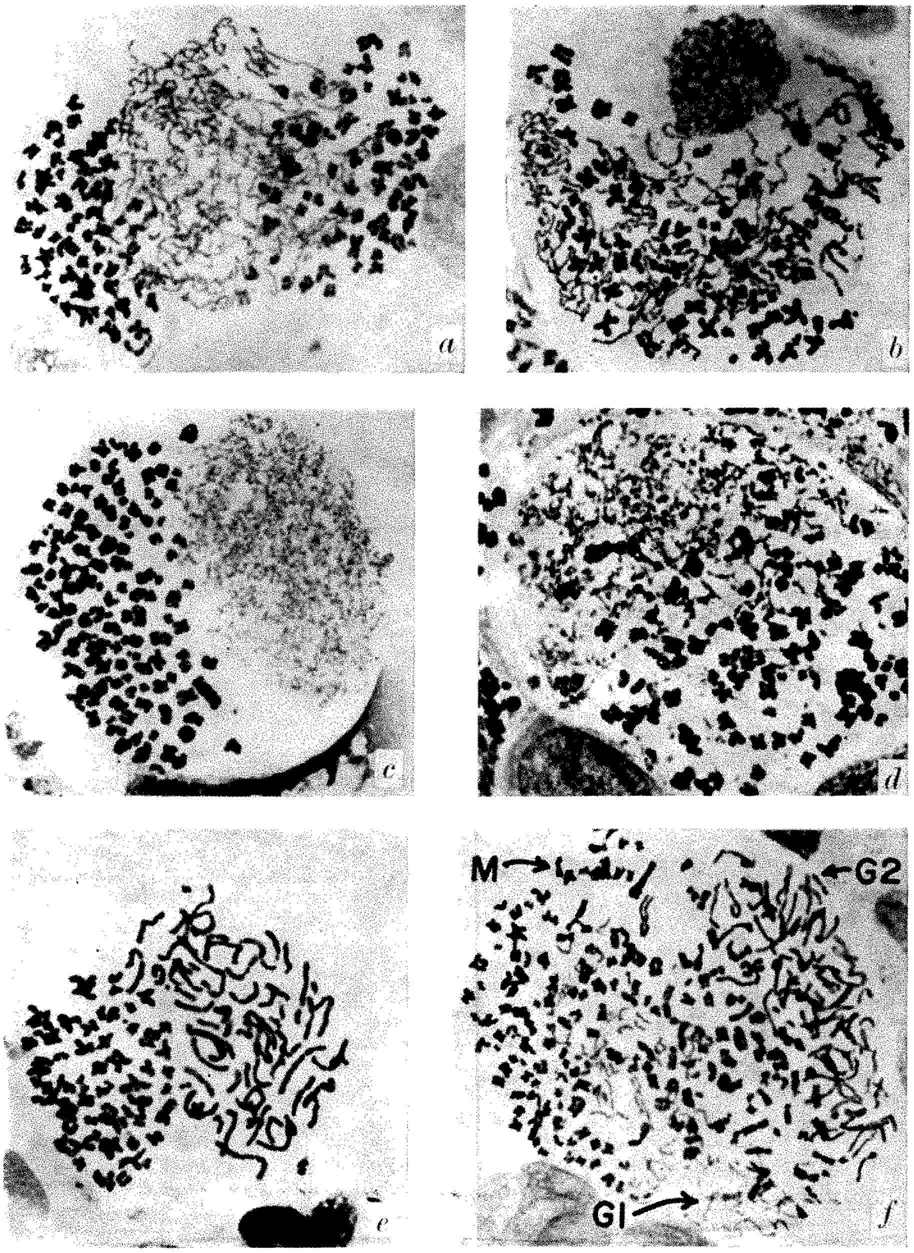

Fig. 3. Premature chromosome condensation in interphase nuclei. *a*, PCC of the *G*1 nucleus in a heterophasic *M*/*G*1 cell at 30 min after the addition of virus. Note the long and slender *G*1 chromosomes with single chromatids. The more condensed chromosomes are from the mitotic cell blocked by colcemide. *b*, Heterophasic cell formed by the fusion of 2*G*1 cells with one mitotic cell. PCC can be observed in the *G*1 nuclei, one of which still shows its nuclear outline. *c*, Premature condensation of the *S* chromatin at 45 min after the fusion between mitotic and *S* phase cells. The chromatin of the *S* nucleus presents a fragmented appearance. *d*, Premature chromosome condensation of a nucleus blocked at the *G*1–*S* boundary by double thymidine treatment. Note that the pattern of condensation is intermediate between that of *G*1 and *S* nuclei. *e*, PCC of the *G*2 nucleus in an *M*/*G*2 heterophasic cell. The more condensed chromosomes are from the mitotic cell treated with colcemide for 19 h. *f*, A heterophasic cell formed by the fusion between *G*1, *G*2 and mitotic cells. Note the differences between *G*1, *G*2 and metaphase (*M*) chromosomes.

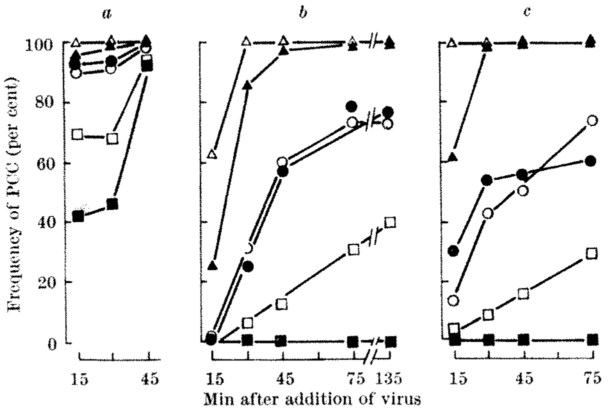

Fig. 4. The frequency of PCC in various classes of heterophasic cells obtained by fusing mitotic cells with (*a*) $G1$; (*b*) S; and (*c*) $G2$ cells as a function of time. △, $3M/(1G1$ or $1S$ or $1G2)$; ▲, $2M/(1G1$ or $1S$ or $1G2)$; ○ $1M/(1G1$ or $1S$ or $1G2)$; ●, $2M/(2G1$ or $2S$ or $2G2)$; □, $1M/(2G1$ or $2S$ or $2G2)$; ■, $1M/(3G1$ or $3S$ or $3G2)$.

interphase chromatin into chromosomal patterns. The $G1$ chromatin condensed into chromosomes with single chromatids, as might be expected from the unreplicated amount of DNA present (Fig. 3*a*, *b*) and the $G2$ chromatin condensed into chromosomes with double chromatids (Fig. 3*e*, *f*). The S chromatin, on the other hand, usually condensed less completely to form a patchwork of large and small fragments interspersed with material that is hardly condensed at all (Fig. 3*c*). In one of the experiments HeLa cells, blocked at the $G1$–S boundary by excess thymidine (2·5 mM) treatment for 18 h, were fused with mitotic cells. The pattern of chromosome condensation in these interphase nuclei was intermediate between the patterns of $G1$ and S phase condensation (Fig. 3*d*). In these nuclei the degree of chromosome condensation is much greater than in S phase nuclei, but these chromosomes have a more fragmented appearance than those from $G1$ nuclei.

DNA synthesis in the prematurely condensed S nuclei was studied at regular intervals by giving a 15 min pulse treatment with ^{3}H-thymidine (1·0 μCi/ml.; 6·7 Ci/mmole) starting 75 min after the addition of virus. This interval was necessary to achieve the induction of PCC in almost all the heterophasic cells. At 75 min the average number of grains per nucleus was 9·0 for the control (mononucleate S phase cells) and 4·3 for the prematurely condensed S nuclei, indicating that the rate of DNA synthesis in the PCC decreased to 48 per cent of that in the control. At 3·25 h the rate of DNA synthesis in the PCC was only 18 per cent of that in the control. The extent of DNA synthesis in the PCC, as measured by grain counts, was related to the proportion of mitotic to S phase nuclei in the heterophasic cells. The greater the ratio of mitotic to S phase nuclei, the greater was the inhibition of DNA synthesis (Table 2). These data demonstrate that the presence of metaphase chromosomes in the same cytoplasm does not immediately stop DNA synthesis in S phase nuclei, even though marked structural changes have been induced in the replicating chromosomes.

The morphological changes observed in the induction of PCC in interphase nuclei parallel exactly those seen in a normal mitosis. A prophase-like pattern was the first indication of division, and this was rapidly followed by dissolution of the nuclear membrane and the greater degree of condensation of the chromatin into whole or "fragmented" chromosomes according to the position occupied by the affected cell in the life cycle at the time of fusion. There was no sign that the induction of chromosome condensation in these cells was accompanied by the appearance of a mitotic spindle.

Induction of PCC as a Function of Dose

The ability of a mitotic cell to induce PCC in an interphase nucleus depends largely on the ratio of mitotic to interphase nuclei in the cell at the time of fusion. The details of this dose response for three different fusions, $M/G1$, M/S and $M/G2$, are shown in Fig. 4. In each case it is clear that the greater the ratio of mitotic to interphase nuclei, the more rapid is the induction of mitosis and the greater is the proportion of cells in the class that shows induction. Compare, for example, the differences in the speed and extent of mitotic induction between the classes $3M/1G1$ and $1M/3G1$, $3M/1S$ and $1M/3S$, and $3M/1G2$ and $1M/3G2$. From this figure it is also evident that PCC could be induced far more readily in $G1$ nuclei than in either S or $G2$ nuclei. After 45 min almost all of the cells containing $1M/3G1$ nuclei were induced (Fig. 4*a*). In comparison, none of the $1M/3S$ or $1M/3G2$ cells were induced even after 75 min (Fig. 4*b*, *c*). In general, the initial frequency of PCC in M/S cells was lower than that in $M/G2$ in almost all the combinations.

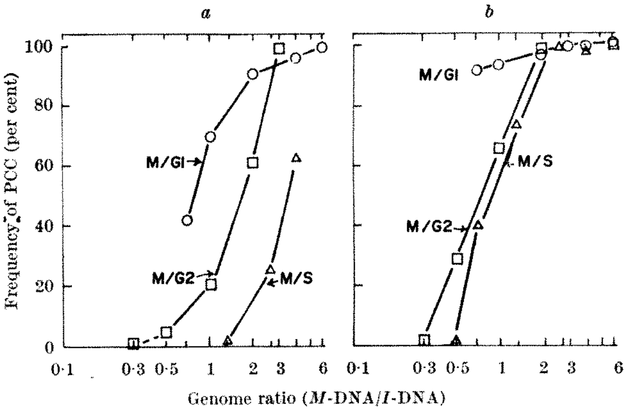

Fig. 5. The frequency of premature chromosome condensation in various heterophasic $M/G1$, M/S and $M/G2$ cells at (*a*) 15 and (*b*) 45 min after addition of virus plotted as a function of the ratio of mitotic DNA to interphase DNA in the cells (genome ratio). The genome ratio is plotted on a log scale. □, $M/G2$ cells; ○, $M/G1$ cells; △, M/S cells.

The data presented so far in Figs. 3 and 4 clearly indicate that the speed and degree of premature condensation of chromatin vary with the position of the interphase cell in the division cycle. The data presented in Fig. 4 were replotted on the basis of the ratios of mitotic to interphase DNA (genome ratios) as shown in Fig. 5. The genome ratios of mitotic DNA to interphase (inducible) DNA were obtained by assuming that a mitotic cell contained two genome units of DNA, while a cell in $G1$ contained one genome unit of DNA. A cell in S phase was assumed to have 1·5 units of DNA, intermediate between $G1$ and $G2$ states.

The derivation of the genome ratios for the various classes of multinucleate cells in each of the three fusions is shown in Table 3. When the frequencies of PCC are plotted against the genome ratios it becomes clear that the susceptibilities for the induction of premature condensation in the $G1$, S and $G2$ nuclei are different (Fig. 5*a*). The induction of PCC in $G1$ nuclei occurs at a smaller genome ratio than in either S or $G2$. At 45 min after the addition of virus the frequencies of PCC in M/S and $M/G2$

Table 2. DNA SYNTHESIS IN PREMATURELY CONDENSED S NUCLEI

	Time (min after addition of virus)	PCC scored $3M/1S$	$2M/1S$	$1M/1S$	$1M/2S$	Total
Per cent incidence of DNA synthesis in PCC (number of cells scored for each class is given in parentheses)	75	20·0 (5)	45·0 (20)	70·6 (48)	100·0 (21)	69·0 (94)
	195	0 (7)	11·0 (18)	44·4 (54)	77·8 (9)	37·0 (88)
Average number of grains per PCC nucleus (number of PCC on which grains were counted is given in parentheses)	75	2·0 (1)	3·1 (9)	4·8 (34)	4·5 (42)	4·3 (88)
	195	0 (0)	1·0 (2)	3·2 (24)	3·5 (14)	3·2 (40)

Table 3. RATIO OF MITOTIC DNA/INTERPHASE DNA EXPRESSED AS GENOME UNITS*

Ratio of mitotic: interphase cells at the time of fusion	$M/G1$ (genome units)	Genome ratio	M/S (genome units)	Genome ratio	$M/G2$ (genome units)	Genome ratio
3 : 1	$3 \times 2/1$	6·0	$3 \times 2/1{\cdot}5$	4·0	$3 \times 2/2$	3·0
2 : 1	$2 \times 2/1$	4·0	$2 \times 2/1{\cdot}5$	2·7	$2 \times 2/2$	2·0
1 : 1	$2/1$	2·0	$2/1{\cdot}5$	1·3	$2/2$	1·0
1 : 2	$2/2 \times 1$	1·0	$2/2 \times 1{\cdot}5$	0·66	$2/2 \times 2$	0·5
1 : 3	$2/3 \times 1$	0·66	$2/3 \times 1{\cdot}5$	0·44	$2/3 \times 2$	0·33

* One genome unit is defined as the average amount of DNA in a $G1$ cell.

cells were similar but still much smaller than in $M/G1$ cells (Fig. 5*b*). Are there any differences in the susceptibility to induction of PCC of $G1$, S, and $G2$ chromatin when the genome ratios

$$\left(\frac{M\text{–DNA}}{I\text{–DNA}}\right)$$

are identical? For example, the genome ratio of the $1M/2G1$ cells is the same

$$\left(\frac{M\text{–DNA}}{I\text{–DNA}}\right) = 1$$

as that of $1M/1G2$ cells, but their degree of susceptibility was significantly different. Differences in the susceptibilities were also observed between $2M/1G1$ and $3M/1S$ cells, both of which have a genome ratio of 4 (Table 3 and Fig. 5*a*). This suggests that the induction of premature chromosome condensation in interphase nuclei does not depend solely on the amount of interphase chromatin to be condensed.

Support for Hypothesis

The results reported here are consistent with the hypothesis that chromosomal condensation can be induced in mammalian cells. The fusion of interphase cells with those in metaphase usually leads to the condensation of the interphase chromatin within a period of 30 to 60 min. The condensation pattern of the interphase chromatin does not occur randomly but follows the sequence of events of a normal mitosis. The final pattern of condensation of the chromatin depends on exactly which stage of interphase the cell occupied at the time of fusion. The chromatin of $G1$ and $G2$ phase nuclei condensed to form chromosomes with single and double chromatids respectively, but the chromatin of S phase nuclei always condensed into a less clearly defined pattern of light and dark staining fragments. In comparison to the S phase nuclei (Fig. 3*c*), the greater degree of chromosome condensation seen in the cells blocked at the $G1$–S boundary by excess thymidine double block (Fig. 3*d*) suggests that the chromatin in these nuclei is probably primed for DNA replication but is not in an active state of DNA synthesis. Removal of the thymidine block leads to the rapid onset of DNA synthesis and incidentally to the characteristic pattern of S phase chromatin condensation of Fig. 3*c*. This difference in the patterns of condensation between S phase chromatin and either $G1$ or $G2$ chromatin suggests that the state of chromatin during the period of DNA replication is different. The physical state of replicating DNA is different from the non-replicating interphase DNA[2,3]. We show here that condensed S phase DNA is still capable of replication, although at a reduced rate. The inhibition of DNA synthesis in the PCC increased with time and with the proportion of mitotic nuclei in the cells. Presumably these two factors influence the rate and degree of condensation of the S chromatin, reducing the number of replicating sites on the DNA double helix.

The dosage effects observed in the induction of PCC may be provisionally interpreted as a titration of the inducer molecules responsible for the condensation of interphase chromatin. G1 phase nuclei are most readily induced into PCC. Even when there were three G1 nuclei and a single mitotic nucleus, at 30 min after completion of fusion 90 per cent of the cells showed complete induction in all the $G1$ nuclei. S and $G2$ phase nuclei were, however, less easily induced and, in a similar 3 to 1 ratio, there was no induction. These differences in the speed and completeness of induction of PCC in $G1$ nuclei on the one hand and the $G2$ or S nuclei on the other suggest that as the amount of DNA per interphase nucleus increases the amount of mitotic inducer must be increased to produce an effect. Even when the mitotic inducer is present in large quantities the induction of S or $G2$ nuclei requires longer periods of time to be accomplished. In multinucleate cells containing a large proportion of interphase to mitotic nuclei there was no mitotic induction. In an attempt to study the effect of PCC on the division of the mitotic cells, interphase cells were fused with mitotic cells obtained by a reversible nitrous oxide block. When the proportion of interphase to mitotic nuclei was 3 : 1 or greater in the heterophasic cells, the mitotic nucleus became pycnotic. This suggests that the presence of a large number of interphase nuclei can inhibit the completion of nuclear division of a metaphase nucleus, and that this interaction is again dose-dependent.

Mitotic : Interphase DNA Ratio

The induction of premature chromosome condensation in interphase nuclei is related, at least in part, to the proportion of the mitotic to interphase nuclei, and therefore to the relative amounts of mitotic and interphase DNA in the cell. It is probable from Fig. 5, however, that the ratio of mitotic DNA to interphase (inducible) DNA is not the only factor to determine whether the interphase chromatin condenses. It is, for example, much easier to induce a given amount of $G1$ chromatin than it is for an equivalent amount of S or $G2$ chromatin when the genome ratio of DNA is the same in either case. This implies that the mitotic inducer has a much more rapid effect on the chromatin of $G1$ than it has on either S or $G2$ chromatin, and this may be related to the physical state of DNA during the $G1$ period.

When $G2$ and S phase cells were fused together the subsequent mitosis of the heterophasic population was asynchronous in 10 per cent of the cases. In these cells the $G2$ nuclei entered mitosis while the "S" nuclei remained in interphase. In some $G2/S$ cells the entry of the $G2$ nucleus into mitosis had induced the premature condensation of the "S" phase chromatin, indicating the presence of a mitotic inducer(s). It is not clear why there was no induction of premature condensation in all the $G2/S$ multinucleate cells where asynchrony in mitosis was observed.

The premature chromosome condensation reported in the present study is similar to the phenomenon first observed by Stubblefield[5] in multinucleate Chinese hamster cells and later termed "chromosome pulverization" by other investigators[6-11]. We consider that "premature chromosome condensation" describes the phenomenon more accurately. Although PCC is most often associated with multinucleate cells it has also been reported in mononucleate permanent cell lines in culture[12,13]. The incidence of premature condensation has been found to increase with an increase in the ploidy of the cell line[13]. Premature entry of nuclei into mitosis, resulting in aberrant nuclear morphology, has also been observed in *Physarum polycephalum*[14,15]. Guttes and Guttes[14] also found that a proportion of these abnormal mitotic nuclei synthesized DNA. These findings are consistent with the observations of Takagi *et al.*[11] and with the results reported here that prematurely condensed S phase nuclei continue to synthesize DNA, although at a reduced rate.

The prematurely condensed chromatin of $G1$ and $G2$ nuclei is not fragmented or pulverized in the sense that myxoviruses normally damage chromosomes[16,17]. The morphology of the condensed *S* phase nuclei does at first seem to resemble virus-inflicted damage in its pulverized appearance. It is possible, however, that the replicative process of the *S* phase chromatin is entirely responsible for the pattern of its condensation. The inactivated virus used for the fusion seems to have no effect on the induction of premature condensation in interphase nuclei[1]. The induction of premature condensation is not accompanied by the appearance of a mitotic spindle, and it therefore remains to be seen how the prematurely condensed chromosomes are distributed during the subsequent division.

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Aurignacian Structural Features and Burials at Cueva Morín (Santander, Spain)

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The discovery of what seems to be the foundation of a hut, an alignment of six post holes, and two graves, one of which contains human remains in a remarkable state of preservation, provides important new information about the earliest Aurignacian occupation of northern Spain.

THE site of Cueva Morín has been known since 1910. Accounts of excavations by the Reverend J. Carballo from 1917 to 1919 and by the Conde de la Vega del Sella between 1918 and 1920 have been published[1,2]. After surveys of prehistoric sites by the Provincial Museum of Santander in 1955 and a sampling programme carried out in Santander and Oviedo in 1962 by one of us (L. G. F., unpublished research), in collaboration with the Seminario Sautuola de Prehistoria y Arqueologia, it was determined that the site would justify renewed excavations. Investigations were begun in 1966 by the Provincial Prehistoric Museum under the direction of J. Gonzalez Echegaray, with the immediate goal of confirming the reported stratigraphy by excavation of the section left along the West cave wall by earlier excavators, and of extending present knowledge of the characteristics of Cantabrian Upper Palaeolithic occupations. Because earlier excavations had stripped the bulk of Upper Palaeolithic occupations from the central and eastern sector of the front of the cave, and Mousterian levels were known to lie near the surface in that area, Cueva Morín seemed an ideal spot to attempt the recovery of large horizontal exposures of Mousterian occupations. In 1968 and 1969 field and laboratory work was carried out under our joint direction. Full accounts of the work will appear elsewhere[3], but we have thought it desirable, in light of the importance of our discoveries, to present a brief résumé of some of the details of our work.

Morín is a critical site for Palaeolithic studies in western Europe and is second to none in Spain in its importance. Among other finds made at the site during 1968 and 1969 are the wall of a Mousterian structure, an extensive Mousterian bone industry (including types strictly analogous with lithic artefacts from Middle Palaeolithic contexts), the first recognized *in situ* Chatelperronian level in the Iberian peninsula, and the subject-matter of this article—an early Aurignacian structural group consisting of an apparent hut foundation, a posthole alignment, and a mortuary complex including two tombs and their appurtenances. Other papers are now in preparation covering the former discoveries, as well as one treating the geological column, by K. W. Butzer.

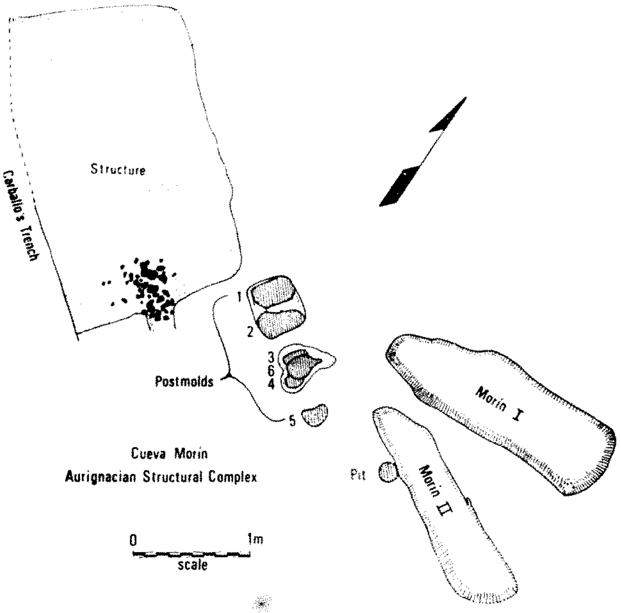

Fig. 1. Aurignacian structural complex, Cueva Morín (drawing).

The Aurignacian stratigraphic sequence at Morin is the only known evidence for the early phases of development of this industrial complex in the Iberian peninsula. It includes six occupation horizons; one assignable to the Aurignacian II stage, two to Aurignacian I and apparently three to Aurignacian "O" (the Perigordian II of Peyrony). The last three levels are designated 8α, 8β and 9, from uppermost to earliest. The characteristic indices[4] for these levels are shown in Table 1. Because the structural complex to be discussed seems to be attributable to level 8α in the general stratigraphy of this site, it is convenient to mention some of the general characteristics of the artefacts from this occupation. The level presents a more typically Aurignacian aspect than do earlier stages in the Aurignacian "O" sequence. Perigordian elements, present in levels 8β and 9, are almost non-existent. There are as many if not more steep (keeled and shouldered) end-scrapers than flat (on flake or blade) endscrapers. Burins are rare and most of them are dihedral, with especially frequent dihedral burins on break. The abundance of *lamelles Dufour* is significant. Judging from the high density of artefacts in this level and the structural complex, occupation during the accumulation of level 8α must have been more intensive than that during the deposition of any other Upper Palaeolithic level at the site.

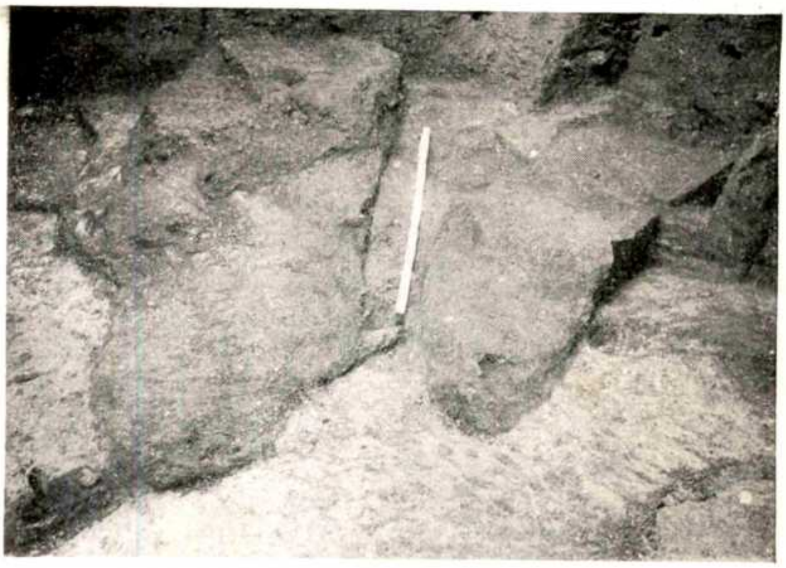

Fig. 3. Mounds atop Morín I and Morín II (seen from west).

The Structural Complex

The stratigraphy of the central and eastern cave sector reveals that the Aurignacian occupants, before the deposition of level 8α or the construction of any of the features, apparently cleared and levelled the cave floor by scraping all accumulated deposits above level 16 (Mousterian of Acheulian tradition) from it. Because level 16 is light in colour, whereas the structural features intrusive into this level are often filled with darker material, those features were first identifiable by the regular dark stains they produced where they contacted the underlying level.

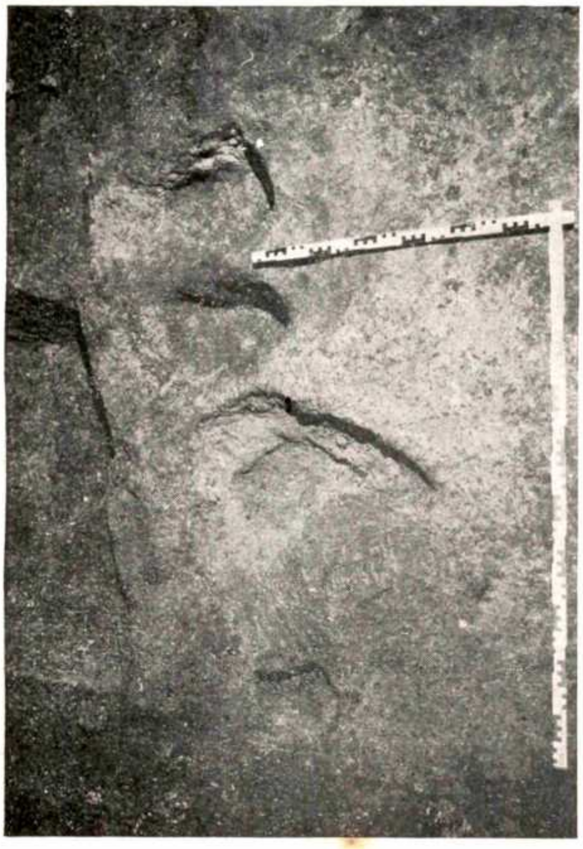

Fig. 2. Posthole alignment and digging stick marks (rule graduated in m).

Table 1. CRITICAL INDICES FOR FIVE AURIGNACIAN OCCUPATIONS AND FEATURES AT CUEVA MORIN

	Levels				Red bed
Index	9	8β	8α	Structure	atop burials
IG	25·3	11·7	24·1	17·2	24·4
IB	9·3	5·8	9·2	8·4	6·6
IBd	5·8	5·8	6·9	4·4	6·1
IBt	0·5	0·0	1·2	1·6	0·0
IGA	9·3	8·8	16·0	4·6	12·3
GA	11·8	11·7	20·6	7·0	14·1
GP	3·4	4·9	0·0	4·7	1·7

The largest feature recovered is the easternmost part of an apparently roughly rectangular excavated depression, measuring approximately 2·6 m on its north-west–south-east axis, and with some 1·7 m of its north-east–south-west axis preserved before a deep trench dug by J. Carballo and extended by the Conde de la Vega del Sella truncates it (Fig. 1). The depth of the standing edges of this pit varies from 27 cm at its northern corner to 3–5 cm along parts of the south-eastern wall. No evidence of postholes or other structural supports was found within or beside the pit, but a distribution of burnt rocks, bone and carbon-rich soil, apparently restricted to a shallow, ovate basin-shaped depression some 35 × 40 cm in diameter, was found inside the south-eastern wall of the structure and is interpreted as a hearth. The basin is joined to a 35 cm long, about 20 cm wide, 6 cm deep trench which slopes upward as it passes to the south-east out of the structure. This part of the feature is interpreted as a draft trench similar to those found attached to some hearths at Corbiac[5]. The large structure did not yield any deliberately constructed superimposed floors and it seems to have been built and used in a single period of activity. This interpretation is partly supported by the fact that stones in the hearth form a continuous distribution from the bottom to the top of the structure fill.

Occasionally, thin laminae of sediment could be traced horizontally over 0·25 m² to 0·50 m² within the fill. These are apparently analogous to laminae produced in fresh deposits added to unexcavated access-ways by the excavators during 1968, when sediment was brought into the cave on workers' feet during the frequent rains. was deposited and

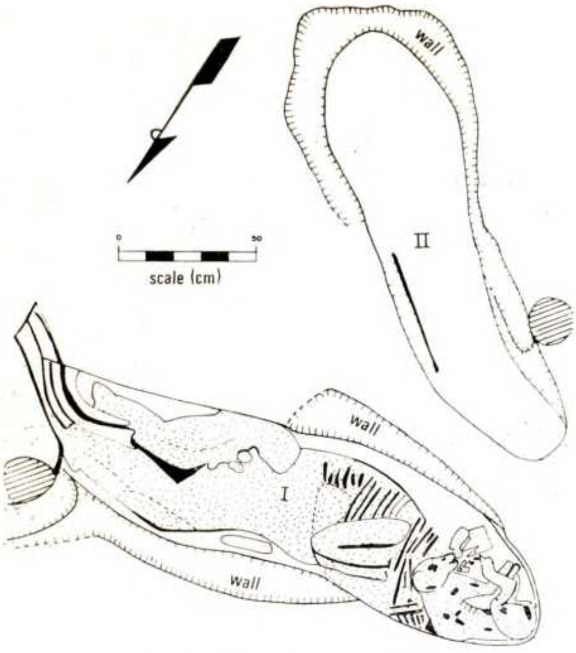

Fig. 4. Morín burials (drawing).

became compacted by trampling as weather dried. Such modern deposition attained a depth of 5 cm during the 1½ month field season in some heavily travelled parts of the site, and if the mechanisms of deposition were similar, it can be suggested that the total depth of laminated Aurignacian structure fill was probably produced during a relatively short period of occupation (perhaps not more than 10 years). The presence of these trampled surfaces and the existence of the hearth suggest that the dugout structure is a hut foundation. It is certainly as large as other features which have been identified as huts or tents by prehistorians, even in its incomplete state of preservation, and similar small structures are known ethnographically as dwellings for nuclear family groups.

Postholes

Starting about 20 cm from the eastern corner of the structure and extending south-east in a rough prolongation of the north-eastern wall of the dugout, an alignment of six pits was discovered in 1968 (Figs. 1 and 2). No wood was recovered from these hollows, but the dark organic stains within them were clearest in a cylinder of earth of less diameter than the pits themselves, while lithic and well preserved bone fragments were abundant surrounding, but not inside, the dark cylindrical stains; this, coupled with the flat-bottomed vertical section of the pits, supports an interpretation of the features as postholes. The character of the stratigraphy of posthole fill and overlying sediments indicates that the (probably wooden) posts were allowed to rot in place, their former cavities being gradually infiltrated with fine sediment. Judging from the size of the cylindrical stains or the diameter of the bases of the postholes, it seems that the maximum possible diameter of emplaced posts could have ranged from 12 to 19 cm.

Details of the structure of the postholes show that posts 1 and 2 (Fig. 1) were set in a single hole and separated by a wall of packed earth fill. Postholes 3 and 4 were also set in a single hole. Only one pile was emplaced in posthole 5. After this post was set, the builders removed posts 3 and 4, replacing them with a single post (No. 6), perhaps to straighten a complex curve in the line formed by the original five posts. No further postholes were recovered during 1969 and it is therefore assumed that the series is complete.

In the process of replacing posts 3 and 4 by post 6, earth removed from postholes 3 and 4 was scooped out of the hole and partially covered posthole 5 (leaving a 12 cm circular area thought to have been caused by the presence at the time of emplaced post 5). The sloping side of postholes 3, 4, 6 produced during this constructional stage bears a series of six well developed shallow arcuate subparallel grooves (Fig. 2). The spacing between adjacent grooves is variable (ranging from about 1·5 cm to about 3·5 cm), as is their depth, which fluctuates between 0·1 and 1·0 cm, and there is no apparent relationship between fluctuations in any set of grooves. These factors suggest that the grooves may have been produced by an excavating instrument with a single, pointed working end. The simplest such implement in modern use is the digging stick. The grooves are not vertical angular striations, but have one wall (the south-western) almost vertical, and the other wall (actually the bottom) inclined at from 70° to 90° to the first. They are all wider towards posthole 5, at the upper end of their trajectory. These observations indicate that the implement was held with point inclined downward, towards the south-west during use, and that the point travelled upward along its trajectory, as would be the case had the tool been used to scoop earth upward out of posthole 4. From the shape of the arcuate grooves and their inclination, one may suppose that the operator was positioned to the north-east of his work, with the implement inclined close to the ground as it would have been had he knelt or squatted by the edge of the pit during work. If the hand that provided driving force was placed high on the shaft of a digging stick used in this fashion, the operator would necessarily have had to be right-handed. The discovery of marks of a Palaeolithic digging implement at Cueva Morín has no parallel of which we are aware in Palaeolithic studies. More such evidence was recovered in 1969.

The Mortuary Complex

South-east of the posthole alignment recovered in 1968, excavations during 1969 revealed the presence of two long tumuli which were recognized as possible human interments. Excavation in the area during the early part of the century was extensive and cut away large sections of both mounds, fortunately without penetrating deeply

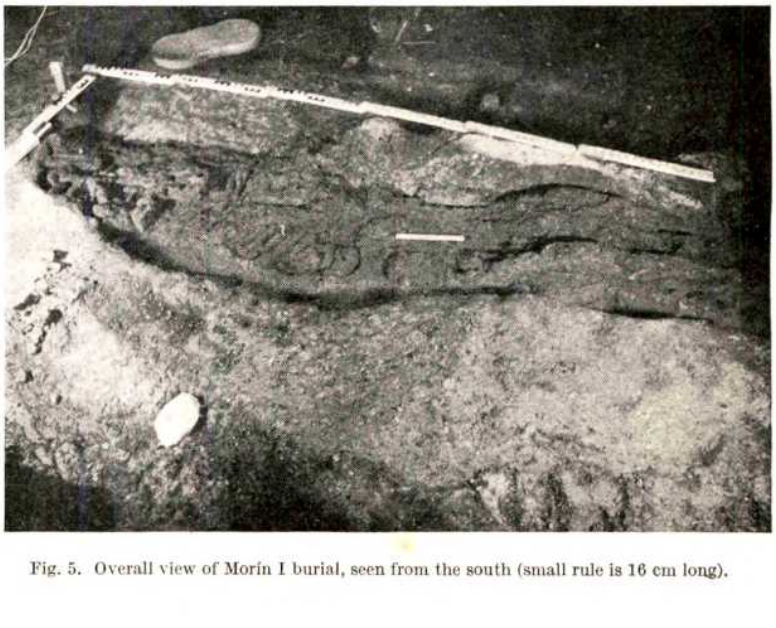

Fig. 5. Overall view of Morín I burial, seen from the south (small rule is 16 cm long).

enough into the strata to disturb the remains of the interments (Fig. 3). The remaining portion of the mound atop Morín I, which was sprinkled with red ochre, measured approximately 115 cm along the axis of the burial, had a maximum width of 75 cm and a greatest height of 44 cm (measured from the surface of the trench containing the burial). The overall length of the mound cannot, of course, be given, but from the length of the preserved mound, coupled with the extent of the burial trench, it is likely to have measured at least 225 cm. Only 57 cm of the mound atop Morín II remained along the burial's long axis; the intact portion was 55 cm wide at its widest point and 40 cm high. The composite length for the standing mound segment and trench was probably about 187 cm. Fig. 1 shows the composite outlines of the intact parts of the tombs and their relation to other structural features.

The tumuli capped two trenches (Fig. 4) which are interpreted as the graves of two individuals. The larger trench, Morín I, has a maximum length of 210 cm and a maximum width of 52 cm, and from foot to head bears S 84° W (mag.). The smaller pit, Morín II, was 168 cm long and 47 cm wide, and bears S 57° E if the heads of the two burials were at the same ends of their respective trenches, or N 57° W if the burials were oriented head to foot. The angle between these bearings is thus either 141° or 39°.

The smaller trench contained no recognizable organic remains other than a dark stain of irregular form in the trench fill. Once the fill had been removed from Morín II, the bottom and sides of the trench proved to be scored with subparallel digging-stick marks like those between postholes 4 and 5.

The larger trench contained human and other remains in a unique state of preservation. As the organic materials in the trench decomposed, their place was taken by fine sediments filtered into the cavities between the remaining organic matter and surrounding deposits, eventually forming a three dimensional mould of the somewhat deformed fleshy parts of the burial and grave goods (Fig. 5). Some (perhaps carbonized) vestiges of organic material are still recognizable in parts of the mould, but on the whole it seems to consist predominantly of fine sediments which are more compact and usually different in colour from the surrounding matrix. The mould is mostly that of a human being of adult stature, lying partly inclined on its left side, in a semi-erect position, with arms positioned before the body and sharply bent upwards at the elbow to bring the hands towards the face. Both the head and the feet are covered by moulds of mortuary offerings. The flesh of the body is represented, and the cadaver has an emaciated appearance which may be the result of deformation by pressures from overlying sediments and decay processes, but may be the result of death by starvation or the wasting effects of disease. The thoracic region is less well preserved than the hips and legs, probably because of relatively rapid decomposition of organs within the body cavity coupled with flattening of the chest and stomach by pressure.

Covering the head of the burial is the exceptionally complete mould of a curled-up small ungulate, lying on its left side, head towards the feet of the burial and front legs tucked between hind legs in the manner in which sheep are still trussed for market (Fig. 6). If this is an adult animal, it must be a roe deer; it could also be a juvenile red deer, caprid or chamois. All these animals are present in the early Aurignacian faunas from Cueva Morín. A number of uninterpretable small moulds were found near the head of this offering and presumably represent other organic grave goods.

The area between the head of the small game animal and the right shoulder of the burial produced several parallel striations which are not a part of the body. They may be remnants of clothing or adornments, but in our opinion their position suggests that they may be the moulds of some binding material such as reeds, hide, or rope used to tie the arms in place in their flexed position during burial. Immediately dorsal to the right upper arm, the ribs of the cadaver form ridges in the sunken flesh. The striations posterior to the ribs are as yet uninterpretable. Part of the body just dorsal to the right elbow was accidentally damaged

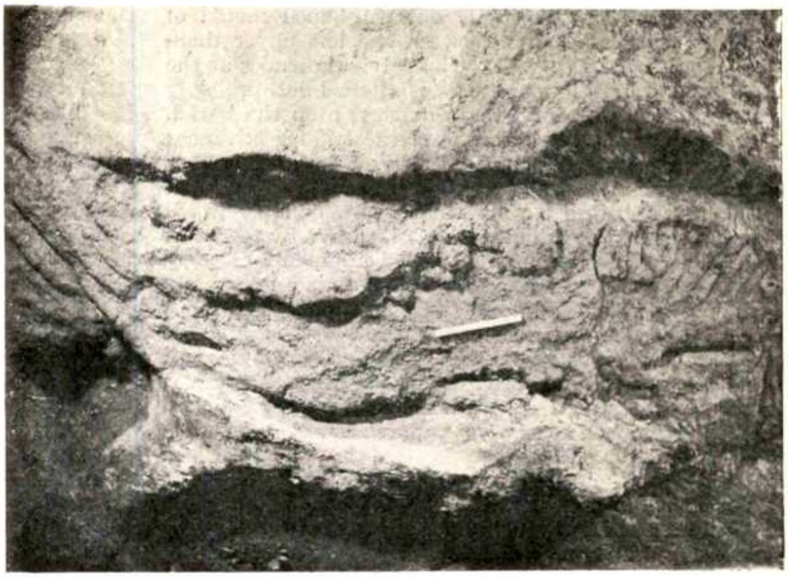

Fig. 7. Detail of pelvic region and legs of Morín I, showing small offering pit at feet and mould of supposed deposit of animal ribs atop foot of trench.

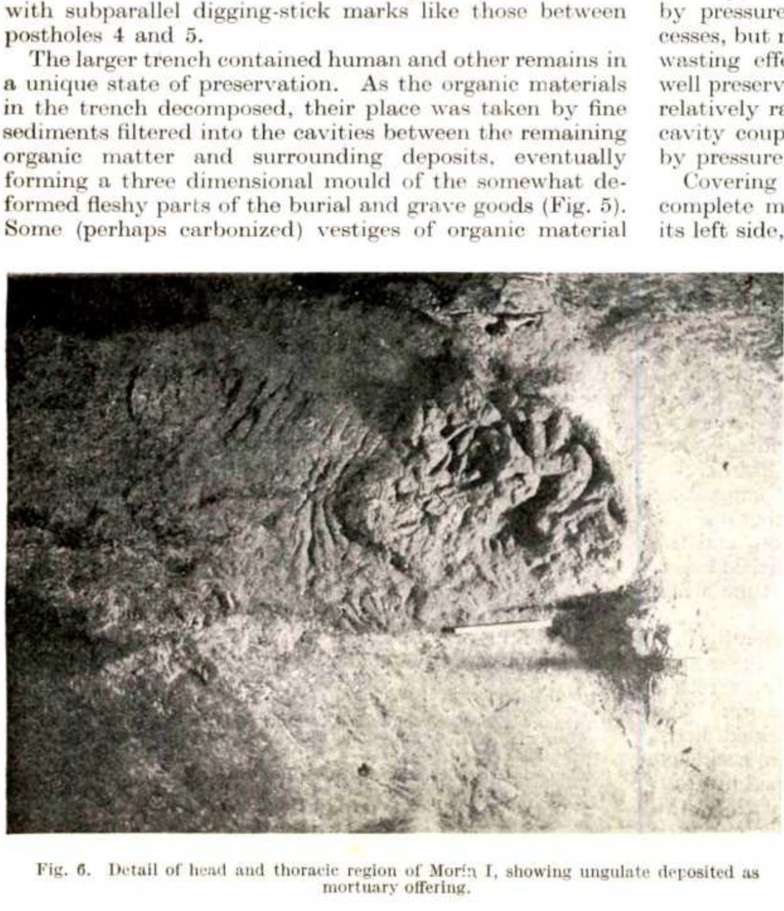

Fig. 6. Detail of head and thoracic region of Morín I, showing ungulate deposited as mortuary offering.

during excavation. A short, sausage-shaped mould of unknown significance lies between the left upper thigh and the grave wall (Fig. 7). The pelvis protrudes at the right hip and both legs are rather flattened out, probably because of the weight of the sediments atop the burial. Both legs are slightly flexed, and the left leg advances before and below the right. The right buttock is very flattened and deformed.

Lying just before the left foot is a small pit which communicates with the interior of the grave by means of a channel. The pit contents yielded fragments of ochre and burnt bone. A similar pit was found in exactly analogous position adjacent to the supposed foot of Morín II; it also communicated with its associated grave and produced similar contents. Beside the offering pit next to Morín II, the negative mould of a Y-shaped wooden or bone object was discovered. A plaster cast removed from the hollow came away containing a well made stone endscraper in a position suggesting that the mould is that of a hafted stone tool. This object may also be related to the mortuary complex. The feet of the Morín I burial are covered by a series of roughly parallel linear moulds, some of which contain bone fragments. These are not restricted to the burial trench but apparently were laid atop the foot of the trench after it had been filled level. They are interpreted as the ribs of a large game animal, but this interpretation is subject to alteration as analysis proceeds. A small fire was lit in a shallow hearth atop the mound covering Morín I and presumably is related to mortuary ceremonial.

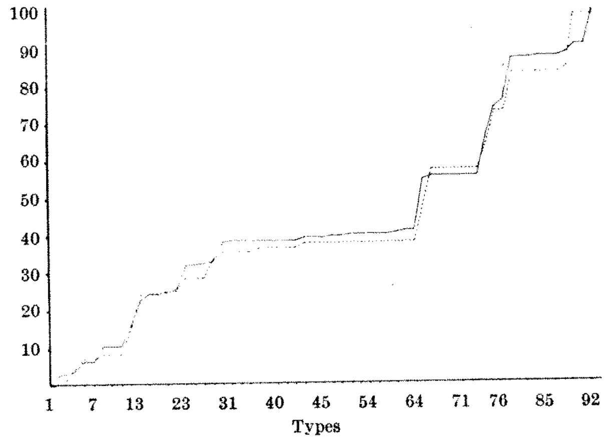

Fig. 8. Cumulative percentage diagram for artefact collections from red bed atop burials (386 pieces) compared with that for level 8α (87 pieces). ———, Red bed; – – – – –, level 8α.

Apparently, while or after the mound was built atop Morín I, the area to its south was deliberately filled with a 20–25 cm deep layer of dark earth. The trench of Morín II was dug through this fill layer, evidence that Morín II is a somewhat later interment than Morín I.

There is, however, stratigraphic evidence suggesting that both trenches and mounds were built during a single construction period of relatively short duration, and the accumulation of the red bed overlying both burials began and continued for an appreciable length of time while both tumuli were completely visible.

Items found in grave fill may be divided generally into two classes—grave furniture and accidental inclusions. No lithic or bone implements recovered from either sepulture can be considered as grave furniture. The trench fills and earth from the mounds yielded lithic artefacts in abundance, but these artefacts were accidentally included in the earth fills which were scraped up from zones of previous occupation. Clods of earth with all the distinctive characteristics of the earlier Mousterian or Acheulian tradition occupation levels at the site were present in both trench fills and mounds. An analysis of the eighty-one retouched pieces from the trench fill of Morín II produces a graph with characteristics to be expected from a mixture of materials from Aurignacian and (mostly Denticulate) Mousterian occupations. Materials from the trench fills and mounds are practically useless in attempts to draw inferences about the material equipment of the tomb builders.

There is no direct connexion between the stratigraphic section in the burial zone and that in the western witness section where the stratigraphic column for Morín is known in most detail. Determination of the approximate stratigraphic position of the burials in our long column must be based on indirect evidence. Because the most recent materials in a fill provide an estimate of the earliest possible date of deposition, the burials are known to be Upper Palaeolithic. Both yielded some pieces out of place in the Morín Mousterian, and the fill of Morín I produced a small, roughly rectangular bone pendant or spatula fragment with tallymarks along both lateral margins. Because the deliberate fill between the trenches and in the mounds contains sediment identical in texture and colour to that in the overlying red bed, it seems that the burials were made after the deposition of a "red bed" was begun somewhere in the cave. No occupation earlier than level 8 is found in sediments with these characteristics. The lithic materials from the red bed overlying the burials produce a cumulative percentage graph (Fig. 8) which is similar to that from level 8α in the stratigraphic column in the western witness section and is quite distinct from the graph for the higher early Aurignacian red level (occupation 6). Thus the burials should be contemporaneous with either level 8β or the earliest phases of deposition of level 8α. Because there seems to be some organic unity in the Aurignacian structural complex as a whole, because the remainder of that complex is quite firmly believed to be contemporaneous with earliest level 8α, and because the burials occupy a stratigraphic position in all ways analogous to the other structural features, we suggest that it is most reasonable to assume that the burials also pertain to level 8α. In any case, no assignment of the burials to any stratum more recent or more ancient than the Aurignacian O levels at Morín can be defended. Carbon-14 dates for the burials may never be determinable, because the mixed nature of burial and mound fills precludes the use of charcoal found within the features for dating purposes. The entire content of the hearth atop Morín I was taken as a carbon-14 sample for assay, but the total amount of charcoal in the sample is small and may be burnt bone rather than burnt wood.

Morín I, jacketed in a fibre glass and plastic box solidly attached to a base of steel, has been removed *en bloc* from the site to the Smithsonian Institition, Washington, DC, for further excavation and the treatment necessary for its permanent conservation. Eventually it will be installed in the Museo Provincial de Prehistoria y Arqueología, Santander.

The burials are the first recognized intentional Palaeolithic interments recovered in the Iberian peninsula, and the structural group is an equally novel find. The discovery of this complex adds an unexpected wealth of detail to knowledge about living conditions and cultural behaviour during the earliest Aurignacian occupation of northern Spain.

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Galactic Cosmic Ray Origin of Li, Be and B in Stars

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A study of the implications of the hypothesis that high energy processes involving cosmic rays acting on the interstellar medium are the sources of the elements Li, Be and B present in stellar atmospheres and in the solar system.

RECENT developments in the observation of L elements (Li, Be, B) in stars and meteorites[1,2] together with progress in studies of the nuclear physics processes involved in the formation of these elements[3,4] make it increasingly hard to hold the view that each star makes its own share of these elements (autogenetic origin). Earlier observational evidence in favour of the autogenetic view has recently either been rejected or made dubious. For instance, the highest abundance values for stellar Li and Be have been shown (Danziger and Vaughan, unpublished, and refs. 5 and 6) to be not larger than the interstellar upper limits ($\mathrm{Li/H} < 10^{-9}$; $\mathrm{Be/H} < 6 \times 10^{-11}$). The missing lithium in the expanding shell of the star T Tauri is still a mystery but probably has nothing to do with the chemical composition of the interstellar medium[7]. Moreover, the variation of lithium and beryllium with spectral class is best explained in terms of destruction mechanisms in the stellar surface (for a review of these questions see ref. 8).

The main difficulty comes from energy considerations. The formation of each L atom requires the acceleration of about 1 erg of fast protons. To account autogenetically for the lithium abundance in T Tauri stars ($\mathrm{Li/H} \simeq 10^{-9}$) the time-integrated amount of energy pumped into particle acceleration must be comparable with the gravitational energy release, implying an unlikely high efficiency for the acceleration mechanism (ref. 9 and unpublished work of Ryter *et al.*).

It therefore seems advisable to consider other sources of the L elements. From the ratios of these elements, one to another, in stars and meteorites, it seems highly probable that the nuclear mechanisms responsible for their generation involve high energy processes (spallation reactions) rather than low energy processes (thermonuclear reactions). Such high energy processes could be due to the action of galactic cosmic rays in their passage through space or at their origin (supernovae explosions, pulsars and so on). In this article we study quantitatively the implications of this hypothesis in particular on the problem of the average intensity of cosmic rays and the solar modulation of the low energy component. We divide the energy range of the galactic cosmic rays into three parts: (1) $E < 5$ MeV nucleon^{-1}, (2) $5 < E < 30$ MeV nucleon^{-1} and (3) $E > 30$ MeV nucleon^{-1}. We find that the lithium abundance is related to the integral flux in region (3) (Φ^{III}) while the boron abundance yields an upper limit on the integral flux in region (2) (Φ^{II}).

Lithium Abundances

The main channels for Li production are: (*a*) p + C,N,O; with a threshold at $E_{\mathrm{p}} \sim 30$ MeV and a mean cross-section of ~ 30 mb, up to the highest energies[3]. (*b*) α + C,N,O; here the centre-of-mass energy is about three times the incident energy per nucleon. The threshold is at ~ 10 MeV nucleon^{-1}. The cross-section is somewhat larger (~ 80 mb) (ref. 10 and Radin, unpublished). (*c*) p,α + Mg, Si; p,α + Fe; the excitation functions rise slowly up to one GeV—above this energy they flatten out reaching values comparable with the corresponding values for C,N,O targets. (*d*) $\alpha + \alpha$; the importance of this reaction was pointed out independently by Hayakawa (unpublished) and Yokoyama (private communication). The threshold is at $E_{\alpha} \sim 12$ MeV nucleon^{-1}. The cross-section rises to ~ 100 mb in the compound-nucleus region (up to a few tens of MeV) and, later on, decreases rather rapidly. A mean value of $\sigma^{\mathrm{II}} \approx 50$ mb is assigned for the region (2), and $\sigma^{\mathrm{III}} \approx 20$ mb at higher energies (region (3)).

Kinetically the L atoms can be formed in two different ways, by the collision of incident light particles on the heavier atoms of the interstellar gas (for instance, fast protons on stationary C, N and O) or the reverse (for instance, fast C, N and O on hydrogen at rest). In the first case the products are essentially at rest, while in the second case they are moving at a velocity comparable with that of the cosmic rays. There are three possibilities for these fast L atoms: (i) deceleration by electron collision, (ii) nuclear destruction by interaction with the interstellar gas, (iii) escape from the galactic disk.

The total inelastic cross-section of L nuclei is approximately 150 mb, corresponding to a mean-free-path of ~ 10 g cm^{-2} against nuclear transformation. This value is comparable with the escape length of galactic cosmic rays out of the galactic disk, as evaluated from various models of cosmic ray diffusion[11].

The deceleration length of fast particles is best studied through the range–energy relationships[12]. For energies below 500 MeV nucleon^{-1} the stopping cross-section of a nucleus of mass A, charge Z and energy per nucleon E (GeV) is conveniently approximated by the expression $\varepsilon = (Z^2/A)[2{\cdot}4 \times 10^{-27} E^{-1}(\text{GeV})]$ cm² GeV, and the range $R = (A/Z^2)[2{\cdot}2 \times 10^{26} E^2(\text{GeV})]$ cm⁻². From this discussion we reach the conclusion that while the L atoms generated by fast protons on stationary atoms are all rapidly thermalized, and become part of the interstellar medium, the L atoms generated by the reverse process have a fate which depends on their initial energy (and hence on the energy of the parent nucleus at the moment of the spallation). L atoms with $E < 0{\cdot}2$ GeV nucleon⁻¹ will effectively be stopped in the galactic gas (and join the initially slow L atoms), while L atoms with $E > 0{\cdot}3$ GeV nucleon⁻¹ will suffer nuclear transformation or escape from the galaxy.

For these initially fast products we define a quantity $f(i+j)$ describing the fraction of L nuclei generated in the process $(i+j)$ which eventually manage to reach thermal velocities in the galaxy. This quantity is, of course, given by the fractional amount of galactic cosmic rays with more than a certain energy, and hence depends on the assumed shape of the cosmic ray energy spectrum. In the spectrum observed at solar minimum the fraction of M ($\varepsilon\Sigma$C to Fe-group) particles with $E < 0{\cdot}25$ GeV nucleon⁻¹ is about 0·2; in a demodulated spectrum the fraction should be larger because the demodulated spectrum is richer in low energy particles than the observed spectrum.

The rate of formation per second of lithium atoms per unit H atom of the galactic gas is given by

$$\begin{aligned} \mathrm{d(Li/H)/d}t = \Phi_{\mathrm{p}}^{\mathrm{III}} & \Bigg\{ \Sigma\sigma(\mathrm{p}+\mathrm{M}\rightarrow\mathrm{Li}) \left[\frac{\Phi_{\mathrm{M}}^{\mathrm{III}}}{\Phi_{\mathrm{p}}^{\mathrm{III}}} f(\mathrm{M}+\mathrm{p}) + \frac{\mathrm{M}}{\mathrm{H}} \right] \\ & + \Sigma\sigma(\alpha+\mathrm{M}\rightarrow\mathrm{Li}) \left[\frac{\Phi_{\mathrm{M}}^{\mathrm{III}}\,\mathrm{He}}{\Phi_{\mathrm{p}}^{\mathrm{III}}\,\mathrm{H}} f(\mathrm{M}+\alpha) + \frac{\Phi_{\alpha}^{\mathrm{III}}\mathrm{M}}{\Phi_{\mathrm{p}}^{\mathrm{III}}\mathrm{H}} \right] \\ & + \sigma^{\mathrm{III}}\,(2\alpha\rightarrow\mathrm{Li}) \frac{\Phi_{\alpha}^{\mathrm{III}}\,\mathrm{He}}{\Phi_{\mathrm{p}}^{\mathrm{III}}\,\mathrm{H}} f(\alpha+\alpha) \Bigg\} \\ & + \Phi_{\alpha}^{\mathrm{II}} \Bigg\{ \Sigma\sigma(\alpha+\mathrm{M}\rightarrow\mathrm{Li}) \left[\frac{\Phi_{\mathrm{M}}^{\mathrm{II}}\,\mathrm{He}}{\Phi_{\alpha}^{\mathrm{II}}\,\mathrm{H}} + \frac{\mathrm{M}}{\mathrm{H}} \right] \\ & + \sigma^{\mathrm{II}}\,(2\alpha\rightarrow\mathrm{Li}) \frac{\mathrm{He}}{\mathrm{H}} \Bigg\} \end{aligned} \qquad (1)$$

where Φ_{p}, Φ_{α}, Φ_{M} are the energy integrated fluxes of particles in space (in the appropriate energy region) and H, He, M are the number densities in the interstellar gas (the most important targets M are C, N, O, Ne, Mg, Si, and iron group nuclei). The factor f (defined previously) is equal to one in the region (2). Let us first compute the value of equation (1) using the flux values observed at solar minimum. Recent measurements are summarized in Table 1.

Table 1. SUMMARY OF RECENT MEASUREMENTS OF GALACTIC COSMIC RAY FLUXES AT SOLAR MINIMUM

	H	He	C,N,O	N	Ne–Si	Fe
Φ^{III} cm⁻² s⁻¹ (E > 30 MeV nucleon⁻¹)	3·6	0·4	25 ($\times 10^{-3}$)	4 ($\times 10^{-3}$)	8 ($\times 10^{-3}$)	2 ($\times 10^{-3}$)
Φ^{II} cm⁻² s⁻¹ (5 < E < 30 MeV nucleon⁻¹)	$\sim 10^{-2}$	$\sim 10^{-3}$				
Abundances relative to H (solar)	1	0·1	15 ($\times 10^{-4}$)	1 ($\times 10^{-4}$)	1·5 ($\times 10^{-4}$)	0·3 ($\times 10^{-4}$)

The proton and helium fluxes at solar minimum are obtained from Freier and Waddington[13] and Comstock, Fan and Simpson[14]. The flux ratio of helium to heavier atoms (known to be almost energy independent down to very low energies) and the recent compilation of Meyer[11] are used to complete the table. Also listed are the solar system abundances (assumed to hold for the interstellar medium).

From the observed galactic cosmic ray spectrum we estimate that $f(\mathrm{M}+\mathrm{p}) \approx f(\mathrm{M}+\alpha) \sim 0{\cdot}2$ while $f(\alpha+\alpha) \sim 0{\cdot}4$ (the lithium energy per nucleon here is only one fourth that of the generating M atom energy).

From Table 1 the fractional contribution of the energy region (2) is negligible and we have

$$\mathrm{d(Li/H)}/\Phi_{\mathrm{p}}^{\mathrm{III}}\,\mathrm{d}t \approx 2 \times 10^{-28}\ \mathrm{cm}^2 \qquad (2)$$

or

$$\mathrm{d(Li/H)/d}t \approx 7 \times 10^{-28}\ \mathrm{s}^{-1} \qquad (3)$$

The L atoms thereby generated become constituents of the interstellar gas, and eventually of the stars formed from it. The photospheric spectral lines of these stars are expected to reflect the galactic gas abundances at the moment of their birth.

Stars are known slowly to destroy their photospheric Li by nuclear reactions in their surface convective zone. The evaluation of their initial Li abundances is best made in young stars (T Tauri type, or members of the Pleiades). To account for lithium abundance in young stars we require

$$\mathrm{Li/H} \sim 10^{-9} \qquad (4)$$

or a total time integrated flux

$$\psi_{\mathrm{p}}^{\mathrm{III}} = \int \Phi_{\mathrm{p}}^{\mathrm{III}}\,\mathrm{d}t = 5 \times 10^{18}\ \mathrm{cm}^{-2} \qquad (5)$$

The necessary average flux over the age of the galaxy ($T_{\mathrm{G}} = 12 \times 10^9$ yr) would be

$$\Phi_{\mathrm{p}}^{\mathrm{III}} = \psi_{\mathrm{p}}^{\mathrm{III}}/T_{\mathrm{G}} = 12\ \mathrm{cm}^{-2}\ \mathrm{s}^{-1} \qquad (6)$$

which is three or four times larger than the present local flux at solar minimum. An analysis of the beryllium abundance yields similar values.

Boron Abundances

The very low threshold for the $^{14}\mathrm{N}+\mathrm{p}\rightarrow{}^{11}\mathrm{C}\rightarrow{}^{11}\mathrm{B}$ reaction ($Q \sim 3$ MeV) can be used to obtain some information on Φ^{II} because no other L isotope can be generated by proton-induced reactions in this energy range. Hence we would expect the $(\Phi^{\mathrm{II}}/\Phi^{\mathrm{III}})$ ratio to have an effect on the $^{11}\mathrm{B}/^{10}\mathrm{B}$ ratio, for instance. We define $^{11}\mathrm{B}^{\mathrm{II}}$ as the abundances of $^{11}\mathrm{B}$ generated in the energy region (2)

$$\begin{aligned} \mathrm{d}\,(^{11}\mathrm{B}^{\mathrm{II}}/\mathrm{H})/\mathrm{d}t = \Phi_{\mathrm{p}}^{\mathrm{II}} & \Bigg\{ \sigma(\mathrm{p}+\mathrm{N}\rightarrow{}^{11}\mathrm{B}) \left[\frac{\mathrm{N}}{\mathrm{H}} + \frac{\Phi_{\mathrm{N}}^{\mathrm{II}}}{\Phi_{\mathrm{p}}^{\mathrm{II}}} \right] \\ & + \Sigma\sigma(\alpha+\mathrm{M}\rightarrow{}^{11}\mathrm{B}) \frac{\Phi_{\alpha}^{\mathrm{II}}}{\Phi_{\mathrm{p}}^{\mathrm{II}}} \left[\frac{\mathrm{M}}{\mathrm{H}} + \frac{\mathrm{He}}{\mathrm{H}} \frac{\Phi_{\mathrm{M}}^{\mathrm{II}}}{\Phi_{\alpha}^{\mathrm{II}}} \right] \Bigg\} \end{aligned} \qquad (7)$$

The average cross-section for $\mathrm{p}+\mathrm{N}\rightarrow{}^{11}\mathrm{B}$ in this region is 120 mb (Epherre, unpublished). For comparison the $(\alpha+\mathrm{M}\rightarrow{}^{11}\mathrm{B})$ in region (3) is ~ 40 mb.

Two observations are of importance here: (*a*) The $^{11}\mathrm{B}/^{10}\mathrm{B}$ ratio in the solar system is much as expected from incident particles in region (3) (refs. 3 and 15). (*b*) The upper limit to the B/Be ratio in the Sun[5] is also close to the spallation ratios in region (3). This leaves little room for

^{11}B formation in region (2). As a rather conservative estimate we take

$$^{11}\mathrm{B}^{\mathrm{II}} < 1/2\ ^{11}\mathrm{B}^{\mathrm{III}} \qquad (8)$$

From equation (7) we obtain

$$\Phi_p^{\mathrm{II}} \leqslant 5\ \mathrm{cm}^{-2}\ \mathrm{s}^{-1} \qquad 5 < E < 30\ \mathrm{MeV} \qquad (9)$$

Abundances of D and ^{3}He

The interaction of galactic cosmic rays with the interstellar gas will generate atoms of D and ^{3}He, chiefly through the p + ^{4}He reaction in region (3). (A smaller amount of D will come from $p + p \rightarrow D + \pi^+$.)

From a ^{4}He cross-section compilation by Meyer[16] we find that a flux capable of generating $\mathrm{Li/H} \simeq 10^{-9}$ will also produce

$$\mathrm{D/H} = 3 \times 10^{-7}$$

and (10)

$$^3\mathrm{He}/^4\mathrm{He} = 3 \times 10^{-6}$$

For comparison the solar system value ($\mathrm{D/H} = 1{\cdot}6 \times 10^{-4}$) is far too high to be explained solely by cosmic rays.

If the solar system value is universal, we could invoke big bang formation (where approximately this value is obtained if 10 per cent of the universal ^{4}He is primordial) or formation by neutron capture in the atmosphere of neutron stars. If the solar system value is local (an upper limit of $\mathrm{D/H} < 3 \times 10^{-5}$ in interstellar space would make us believe that it is), we may have to invoke non-nuclear processes such as the preferential escape of the lighter particles during the evaporation of the early solar nebula.

Because the helium generated by proton thermonuclear reactions has an isotopic ratio $\sim 10^{-3}$, from equation (10) one does not expect cosmic rays to make an important contribution to the ^{3}He production. The initial meteoritic value ($\sim 10^{-4}$) is indeed typical of hydrogen burning (perhaps even somewhat smaller).

Lithium Isotopic Ratio

The ^{7}Li/^{6}Li ratio has been measured in a number of stars[1] and the values range from 2 (the formation ratio) to larger than 10. Preferential destruction of ^{6}Li is easily explained by thermonuclear fusion in the stellar convective zone during the pre-main sequence or the main sequence stages (although some difficulties remain with the ^{9}Be/^{7}Li/^{6}Li ratios).

In the solar system (Earth and meteorites) the isotopic ratio is 12·5. For various reasons it seems difficult to explain the ^{6}Li destruction by thermonuclear reactions in the surface convective zone of the Sun[15,17]. A more promising attempt would involve preferential destruction of ^{6}Li by (p,α) reactions in the matter which was thrown off the Sun to form the solar system. At present, however, it is fair to say that we have no satisfactory explanation of the lithium isotopic ratio in meteorites.

Discussion

To obtain the value of Φ^{III} or the upper limit to Φ^{I} we have neglected lithium destruction by stars. Schmidt's model of galactic evolution[18] predicts an overall destruction rate which is too small to affect our conclusions: according to his calculations more than 50 per cent of the galactic gas has never been part of a star.

The difference between the observed flux above 30 MeV (Φ_p^{III}(obs)) and the time average flux deduced from L element abundances (Φ_p^{III}) can be ascribed to three possible effects.

(*a*) Solar modulation. The effect of the "residual" modulation at solar minimum has been calculated by a number of authors from their favourite version of the modulation theory. The ratio $\Phi_p^{\mathrm{III}}/\Phi_p^{\mathrm{III}}$(obs) is found to be $\sim 1{\cdot}2$ (ref. 19), $\sim 1{\cdot}5$ (ref. 20), ~ 7 (ref. 21) and ~ 20 (ref. 22). Clearly a satisfactory theory applicable down to 30 MeV is yet to come. The L element abundance can, in fact, be used to obtain a limit on the solar modulation. In doing so one must realize that by inserting in equation (1) the demodulated flux instead of the observed flux, one must also include the effect of the modulation on f (see the discussion following the definition of f). Indeed, the solar influence exerts itself mostly on the low energy nuclei. The fraction of decelerating L nuclei should accordingly be increased. As a result, a time integrated flux $\sim 3 \times 10^{18}$ particles cm^{-2} would probably be enough to generate the correct amount of L nuclei.

(*b*) Spatial clumpiness of the high flux regions. The fluxes in the vicinity of the sources (supernovae, pulsars, flare stars, and so on) are likely to be of much higher intensity than in an average galactic location. (The L atoms would later diffuse in space.) Such hot spots could in principle be detected from the accompanying X- and γ-ray emission.

(*c*) Time variation. The time constancy of galactic cosmic rays has been verified only for the last billion years or so (8 per cent of the galactic life). We have serious reasons to believe that star formation and nucleosynthetic processes were much stronger in the early days of the galaxy[23]. A similar evolution could well have taken place for the galactic cosmic rays. It may indeed turn out that most of the L elements were made during this initial epoch of strong stellar activity.

The analysis of the ^{11}B/^{10}B ratio shows that the flux of galactic cosmic rays is not heavily concentrated in the low energy region, at least for energies as low as 5 MeV. This statement has a bearing on the problem of the heating of HI regions and on the emission of galactic gamma rays. These questions will be treated later.

We thank A. G. W. Cameron for pointing out problems in the efficiency of autogenetic acceleration mechanisms, B. Peters (who first suggested to us the possible role of the low energy cosmic rays in generating the L atoms), Yash Pal for discussions of cosmic ray phenomena, and G. R. Burbidge, D. D. Clayton and J. P. Meyer for comments.

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LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Planetary Companions of Pulsars

THE recent observation of a "quasi-sinusoidal" period variation in the pulsar NP 0532 has been attributed to perturbations produced by a planetary companion with an orbital period of about three months and a minimum mass of 1·0 $M_\oplus$ assuming the mass of the pulsar to be 1 $M_\odot$ (ref. 1). It is therefore desirable to investigate the orbit of a planet after a supernova explosion of the central star, and the effect of the new orbit on the observed period of the pulsar.

I will assume that before the explosion all planets were in circular, coplanar orbits. The evolution of the orbit is caused chiefly by the loss of mass by the central star and not from the impact of the expanding shell which for an inelastic collision gives the planet an outward velocity

$$\Delta V_p = V_s \frac{\Delta M}{m} \left(\frac{S}{2r_0}\right)^2 \tag{1}$$

A shell velocity $V_s = 10^4$ km s^{-1} and mass $\Delta M = 1$ $M_\odot$ with planet parameters $m = 1$ $M_\oplus$, $r_0 = 0{\cdot}4$ AU, and $S = R_\oplus$ gives $\Delta V_p = 1{\cdot}5$ km s^{-1}. Because the orbital velocity is about 50 km s^{-1} this only produces an eccentricity $e = 0{\cdot}03$. Colgate[2] has recently discussed the conditions necessary for a supernova blastwave to eject a planet.

Because $V_s \gg V_p$ we can assume, for dynamical purposes, that the loss, ΔM, in the original stellar mass, M_0, is instantaneous. This loss decreases the gravitational energy binding a planet to the central star without affecting its kinetic energy. From these considerations we can easily show that the fractional increase in the semi-major axis of all the planets is given by

$$(r/r_0) = 1/2 \left[\frac{M_0 - \Delta M}{1/2\, M_0 - \Delta M}\right] \tag{2}$$

We note that if $\Delta M \geq 1/2\, M_0$ these planets escape. Thus if $\Delta M = 1$ $M_\odot$ in NP 0532, M_0 was at least 2 $M_\odot$, which gives it a main-sequence spectral class of late B or earlier and makes it a population I supernova.

Blaauw[3] found that for a double-star system to dissociate due to a supernova explosion requires that ΔM be greater than half the combined mass of the two stars or $\Delta M \sim M_0$ if the stars have similar masses. In view of the observed high frequency of binary stars and their more stringent dissociation requirements compared with planetary systems, it is curious that no pulsar has been observed with a stellar companion.

While the angular momentum of the planet is unaffected by the explosion, the increase in the semi-major axis causes its eccentricity to assume a new value

$$e = \left[1 - 2\left(\frac{1/2\, M_0 - \Delta M}{M_0 - \Delta M}\right)\right]^{1/2} \tag{3}$$

or

$$\Delta M/M_0 = e^2/(1+e^2) \tag{4}$$

The eccentricity is high for any reasonably assumed $(\Delta M/M_0)$; for example, $e = 0{\cdot}58$ if $(\Delta M/M_0) = 1/4$ and $e = 0{\cdot}77$ if $(\Delta M/M_0) = 3/8$. As e can be determined by observation in a fashion analogous to the determination of the elements of a spectroscopic binary orbit it will be possible to calculate $(\Delta M/M_0)$ for NP 0532.

If there are several planets in the system the large eccentricities result in the overlapping of many orbits if the spacings of their semi-major axes are similar to those in the solar system. This unstable situation will start a new epoch of active dynamical evolution which may lead to the ejection of many of the less massive planets before the survivors settle into new orbits which minimize the effects of their mutual perturbations. The properties of these final orbits are probably similar to those found by myself[4-6] in computer-simulated planetary systems evolved from arbitrary initial conditions even though the initial eccentricities of the post supernova orbits are probably somewhat higher than those that I used. The computer experiments indicated that the four Jovian planets took about 10^5–10^6 yr to evolve into stable orbits under the influence of their mutual perturbations. For less massive planets of the terrestrial type with semi-major axes as small as 0·4 AU the strong gravitational coupling to the central star combined with weak mutual perturbations make it unlikely that their orbits can be radically altered in time scales of less than 10^9 yr. Thus the orbit of the observed planetary companion of NP 0532 may have remained nearly unchanged since the explosion.

It may be possible to attribute the apparently abrupt change of period observed in NP 0532 to the perturbations produced by its planetary companion*. The observed rotational period, P, of the pulsar due to its motion around the centre of mass of the star–planet system is given closely by

$$\frac{\mathrm{d}(P/P_0)}{\mathrm{d}t} = \frac{1}{c}\left(\frac{\mathrm{d}V}{\mathrm{d}t}\right) \tag{5}$$

where P_0 is the observed period in the absence of orbital motion and V is the radial velocity of the pulsar due to its orbital motion. It is evident that the (P/P_0) and V curves are identical except in scale. From the classical equation for the radial velocity of a spectroscopic binary[7] and equation (5) we easily find that

$$\frac{P - P_0}{P_0} = \frac{V}{c} = \left[\frac{(2\pi G)^{1/3}\, m \sin i}{c(1-e^2)^{1/2}\, \tau^{1/3}\, (M+m)^{2/3}}\right] (\cos(\nu + \omega) + e \cos \omega) \tag{6}$$

where τ is the orbital period, $M = M_0 - \Delta M$, i is the inclination of the orbit plane to the line of sight, ω is the longitude of periastron measured from the ascending node, and ν is the true anomaly which is related to the time, t, since periastron passage by the classical equations

$$\tan (1/2)\, \nu = \left(\frac{1+e}{1-e}\right)^{1/2} \tan (1/2)\, E \tag{7}$$

and

$$2\pi\left(\frac{t}{\tau}\right) = E - e \sin E \tag{8}$$

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* After the draft of this paper had been completed, a preprint of a paper by F. C. Michel with the same suggestion was brought to my attention.

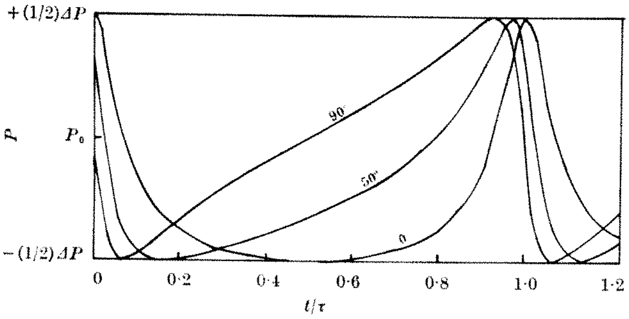

Fig. 1. Variation in the observed period of a pulsar due to its orbital motion relative to the centre of mass of the planet–pulsar system. The cases shown are for values of ω of 0°, 50°, and 90° and $e=0·6$.

For mass in $M_\odot$ and τ in years the maximum variation in the period of a pulsar due to orbital motion is

$$\frac{\Delta P_{\max}}{P_0} = \left[\frac{1·99\times 10^{-4}\, m \sin i}{(1-e^2)^{1/2}\tau^{1/3}\,(M+m)^{2/3}}\right] \qquad (9)$$

Fig. 1 shows, for illustration, the time dependence of P for $e=0·6$ and various values of ω. For large ω and e the minimum time, $\Delta t_{\min}$, required to go through the maximum period range, $\Delta P_{\max}$, is much less than 0·5 τ. In Table 1 I have tabulated $(\Delta t_{\min}/\tau)$ as a function of e and ω. If $(\Delta t_{\min}/\tau)$ is small the pulsar will be observed to undergo an apparently abrupt increase or decrease in period depending on the orientation of the planetary orbit if it is not observed with sufficiently high time resolution. Both radio[8] and optical[9] observations have recorded a decrease in the period of NP 0532 of $(\Delta P/P_0)=2·3\times 10^{-9}$ within the fifteen day interval between October 8 and October 23, 1969. Because this is about 0·16 of the orbital period of the planet, it could be explained by the planet being in an orbit having e as low as 0·55 if $\omega=90°$ or $e=0·7$ if $\omega=45°$, the average ω for randomly oriented orbits. From equation (9) we find that the minimum m required to explain the observed $(\Delta P/P_0)$ is 1·3 $M_\oplus$ if $e=0·8$ and $\tau=0·25$ yr, which is very close to the m required to explain the quasi-sinusoidal variation in the period of the pulsar. If the abrupt drop in period was caused by the planetary companion a similar drop can be expected towards the end of January due to the three month period of the planet.

Table 1. MINIMUM TIME IN UNITS OF THE ORBITAL PERIOD BETWEEN THE OBSERVED MAXIMUM AND MINIMUM ROTATIONAL PERIODS OF A PULSAR DUE TO PLANETARY MOTION

e	0°	10°	20°	30°	40°	50°	60°	70°	80°	90° ω
0·00	0·500	0·500	0·500	0·500	0·500	0·500	0·500	0·500	0·500	0·500
0·05	0·500	0·494	0·489	0·484	0·480	0·476	0·472	0·470	0·469	0·468
0·10	0·500	0·489	0·478	0·468	0·459	0·451	0·445	0·440	0·437	0·436
0·15	0·500	0·483	0·467	0·452	0·438	0·427	0·417	0·410	0·406	0·405
0·20	0·500	0·477	0·456	0·435	0·417	0·402	0·390	0·381	0·375	0·374
0·25	0·500	0·471	0·444	0·419	0·396	0·377	0·362	0·351	0·345	0·343
0·30	0·500	0·465	0·432	0·402	0·375	0·352	0·335	0·322	0·314	0·312
0·35	0·500	0·459	0·420	0·384	0·353	0·327	0·307	0·293	0·285	0·282
0·40	0·500	0·452	0·406	0·365	0·330	0·302	0·280	0·264	0·255	0·252
0·45	0·500	0·445	0·392	0·346	0·307	0·276	0·252	0·236	0·227	0·224
0·50	0·500	0·437	0·377	0·326	0·283	0·250	0·225	0·208	0·199	0·196
0·55	0·500	0·428	0·361	0·304	0·258	0·223	0·198	0·181	0·172	0·168
0·60	0·500	0·418	0·343	0·281	0·232	0·197	0·171	0·155	0·145	0·142
0·65	0·500	0·407	0·324	0·256	0·206	0·169	0·145	0·129	0·120	0·118
0·70	0·500	0·394	0·301	0·229	0·177	0·142	0·119	0·104	0·097	0·094
0·75	0·500	0·378	0·275	0·199	0·148	0·115	0·094	0·081	0·074	0·072
0·80	0·500	0·358	0·244	0·166	0·117	0·088	0·070	0·059	0·054	0·052
0·85	0·500	0·330	0·205	0·128	0·085	0·061	0·047	0·039	0·035	0·034
0·90	0·500	0·289	0·154	0·086	0·053	0·036	0·027	0·022	0·019	0·019
0·95	0·500	0·214	0·085	0·039	0·021	0·014	0·010	0·008	0·007	0·007

It is desirable to look for longer-period semi-sinusoidal variations in NP 0532 produced by planets in more distant orbits. A planet with a mass equal to that of Jupiter in an orbit with $e=0·8$, $\omega=70°$ and located 5 AU from the pulsar could produce a period change $(\Delta P_{\max}/P_0)=1·5\times 10^{-7}$ within an eight month interval. In the theory of planet formation which I suggested[4] it is doubtful whether Jovian-type planets can form around main sequence stars that are very much more luminous than the Sun as presumably was the case for NP 0532; on the other hand, it would be surprising if no more terrestrial-type planets are found in the system.

Even with a planet of Jovian mass it is difficult to account for the period change $\Delta P/P_0=1·5\times 10^{-6}$ observed within a seven-day interval in PSR 0833–45 (refs. 10 and 11). A suggested[12] decrease in the moment of inertia following a stellar quake may be a reasonable explanation for a change of this magnitude. More observations with finer time resolutions are needed to help interpret these sudden changes in period.

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Plasma Turbulence as a Mechanism of Pulsar Radiation

RADIO signals from pulsars are characterized by (1) high intensity, with brightness temperatures of the order of 10^{21} K (ref. 1); (2) pronounced (mostly linear) polarizations with, in the case of PSR 0833 (ref. 2), a monotonic sweep of the polarization angle during each pulse; (3) narrow pulse widths (Δt) compared with the repetition period (P); and (4) optical, X-ray and radio pulse widths which are virtually the same[3]. This report is about a pulsar model in which anisotropic plasma turbulence, produced by the onset of a two-stream instability near the light cylinder of a rotating magnetic star, is the chief radiation mechanism. The model offers a quantitative, self-consistent account of features (1)–(3) for quite different pulsars, and a qualitative explanation of feature (4).

We consider a plasma with electron density n near the light cylinder corotating with the magnetic star. The magnetic lines of force passing through the plasma penetrate the light cylinder and may reach the supernova shell or interstellar space[4]. We also assume a (relativistic) electron beam with density n_b and velocity $\mathbf{v}$ along the magnetic lines of force, so that this is a typical case of a two-stream instability in the plasma. The plasma will thus be strongly turbulent, with the static form factor $S(\mathbf{k})$ (ref. 5) of the density fluctuations in the wave-vector space enormously enhanced over thermal levels.

Because of the anisotropy caused by the magnetic field $\mathbf{B}$ and the streaming electrons $(\mathbf{v}\|\mathbf{B})$, we expect enhanced electromagnetic radiation associated with the plasma turbulence. The power $\mathrm{d}^2\mathcal{P}/\mathrm{d}o\,\mathrm{d}\omega$ of such radiation in the frequency interval $\mathrm{d}\omega$ emitted through a unit normal area into a solid angle do in the direction of **k** is (ref. 6 and my unpublished work with Starr)

$$\frac{d^2\mathcal{P}}{do d\omega} = \frac{ne^2}{2\pi^2 c^2}\,\omega^3 \int_0^\infty dk\; \frac{|\varepsilon_1|^2 + |\varepsilon_2|^2}{k^3}\, S(\mathbf{k})\delta(\omega - \mathbf{k}\cdot\mathbf{v}) \qquad (1)$$

where

$$\varepsilon_1 = \frac{\omega_p{}^2 \Omega^2}{\omega^2(\Omega^2 - \omega^2)} \sin\theta\cos\theta \qquad (2)$$

$$\varepsilon_2 = i\,\frac{\omega_p{}^2 \Omega}{\omega(\Omega^2 - \omega^2)} \sin\theta \qquad (3)$$

$\omega_p = (4\pi n e^2/m)^{1/2}$, $\Omega = eB/mc$, and θ is the angle between **k** and **B**. In equations (2) and (3) we have neglected the contribution of the streaming electrons by assuming $n \gg n_b$.

The detailed structure of the spectral function $S(\mathbf{k})$ should be determined from the solution of a non-linear self-consistent integral equation[7]. Here it suffices to note that the spectrum is broad enough to cover the observed range of radio frequencies and that it takes on a maximum value at about $k_m = \omega_p/v$ when **k** is parallel to **B**. The properties of pulsar radiation can now be correlated with equation (1).

(1) The beam-plasma system can inherently sustain unstable collective modes down to $k = 0$. The level of turbulence in such a system is limited only by particle collisions or by the effect of the finite system dimension. For plasmas surrounding rotating magnetic stars, we expect collisional effects to play the chief part. The average level of turbulence $\langle S \rangle$ may be estimated to be (ref. 7 and my unpublished work)

$$\langle S \rangle \simeq n l^2 k_m / 2\pi K^2 \qquad (4)$$

where l is the mean free path and K is a maximum perpendicular wave number for the collective modes to be relatively undamped excitations and n/K^2 measures a line density of the electrons involved in the two-stream instability. When $\Omega^2 \gg \omega_p{}^2$, $K^2 \simeq (m\Omega^2/\varkappa T)$; and when $\Omega^2 \ll \omega_p{}^2$, $K^2 \simeq (m\omega_p{}^2/\varkappa T)$, where $\varkappa$ is the Boltzmann constant and T is the electron temperature. According to equation (4) (and equation (6) later) there should be quite different turbulent behaviour for "fast" as against "slow" pulsars, because a fast pulsar has a large B at the light cylinder. B differs so much that it is reasonable to assume $\Omega^2 \gg \omega_p{}^2$ for fast pulsars such as NP 0532 and PSR 0833 and $\Omega^2 \ll \omega_p{}^2$ for slow pulsars such as CP 0328 and CP 1919, an assumption which will be shown to be self-consistent.

(2) From equations (2) and (3), we note that

$$|\varepsilon_1/\varepsilon_2| = |\Omega/\omega| \cos\theta \qquad (5)$$

Thus, when Ω is much greater than the relevant frequencies of the radio emission, we have $|\varepsilon_1/\varepsilon_2| \gg 1$ and the radiation is linearly polarized in the direction perpendicular to **k** on the plane made by **k** and **B**. When $\Omega \ll \omega$, $|\varepsilon_1/\varepsilon_2| \ll 1$ and the radiation is linearly polarized in the direction perpendicular to the plane of **k** and **B**. The **k** direction is fixed in the line of sight—the **B**–**k** plane (that is, the **v**–**k** plane) rotates around this line as the star rotates. Thus the sweep of the position-angle is explained. We also note in equation (5) that the degree of linear polarization depends on the frequency of observation. In the case of PSR 0833 (where $\Omega \gg \omega$), lower frequency radiation should be more linearly polarized[2].

(3) Radiation from the anisotropic plasma turbulence due to the two-stream instability has exactly the property of emission in a pencil or fan beam which seems necessary to explain the pulsations. Turbulence is strongest when **k** is parallel to **B**; it decreases sharply as **k** acquires a component perpendicular to **B**, either because of the finite Larmor-radius effects (when $\Omega^2 \gg \omega_p{}^2$) or because of Landau damping (when $\Omega^2 \ll \omega_p{}^2$). The angle θ_m of the "cone" within which the wave vectors of the turbulent fluctuations are confined may then be estimated from such consideration as

$$\theta_m \simeq \begin{cases} \tan^{-1}[\Omega(mc^2/E_p)(m/\varkappa T)^{\frac{1}{2}}(v/\omega_p)] & (\text{for } \Omega^2 \gg \omega_p{}^2) \\ \tan^{-1}[\omega_b(mc^2/E_p)^{\frac{1}{2}}(m/\varkappa T)^{\frac{1}{2}}(v/\omega_p)] & (\text{for } \Omega^2 \ll \omega_p{}^2) \end{cases} \qquad (6)$$

where $\omega_b = (4\pi n_b e^2/m)^{\frac{1}{2}}$, and $E_p = c(p^2 + m^2c^2)^{\frac{1}{2}}$ is the energy of a streaming electron with the momentum p. The ratio $\Delta t/P$ must then be equal to or somewhat smaller than θ_m/π.

Table 1. OBSERVED OR ESTIMATED QUANTITIES

	Period P(G)	Width Δt(ms)	Radio power $E[10^{-26}$ J m^{-2} Hz$^{-1}]$	Distance D[kpc]	Electron density n[cm^{-3}]	Electron temperature T[K]
NP 0532	0·033	9·5	0·2 (at 196 MHz)	1·7	5×10^{10}	10^6
PSR 0833	0·089	2	0·2 (at 408 MHz) 0·1 (at 1,420 MHz)	0·41	5×10^{10}	10^6
CP 0328	0·715	7	1 (at 408 MHz) 0·3 (at 1,420 MHz)	0·48	10^{10}	2×10^5
CP 1919	1·34	40	0·2 (at 408 MHz)	0·23	10^{10}	7×10^4

Table 2. COMPUTED QUANTITIES

	Magnetic field of emitting region $B(g)$	Surface magnetic field $B_0 = B(L/10^6)^3$	$\beta = \frac{n\chi T}{B^2/8\pi}$	Momentum of beam particle $p(mc)$	$\left(\frac{n_b}{n}\right)\left(\frac{mc}{p}\right)$
NP 0532	10^4	4×10^{10}	2×10^{-6}	$1{\cdot}6 \times 10^5$	—
PSR 0833	2×10^3	$1{\cdot}6 \times 10^{11}$	5×10^{-5}	$2{\cdot}2 \times 10^5$	—
CP 0328	10	4×10^{11}	7×10^{-2}	—	5×10^{-8}
CP 1919	2·7	$7{\cdot}1 \times 10^{11}$	3×10^{-1}	—	$1{\cdot}7 \times 10^{-7}$

Tables 1 and 2 summarize the comparison with observations. In Table 1, P, Δt and E are from raw data[1], and in column 4 we use the distance estimates from ref. 8. Each value of E may then be converted into the radiation intensity at the source by assuming that the emitting region has radius, $L = cP/2\pi$. The electron densities (column 5) are chosen so that the plasma frequencies fall within the ranges of the observed radio frequencies and the values reflect our effort to take account of the pulsar radio spectrum[1]. The electron temperatures (column 6) are the estimated surface temperatures ($\sim 10^6$ K) of neutron stars, and the smaller values for the slow pulsars are from the inequality $n\varkappa T/(B^2/8\pi) < 1$, a condition for a magnetically controlled plasma. These choices are physically consistent with the idea of plasma cooling as L increases for a slow pulsar. The Coulomb mean-free-paths are then computed.

The numerical values are substituted into the expressions for radiation intensity and pulse width, thus determining the strength of the magnetic field in the emitting region (column 1 of Table 2). Self-consistency of our model would then demand that those magnetic fields represent the fields appropriate to the vicinity of the light cylinder. To check this, we compute $B_0 = B(L/10^6)^3$ in column 2. This quantity should give an estimate of the strength of the surface magnetic field, assuming a dipole field and a stellar radius of about 10 km. The calculated surface fields B_0 agree almost within an order of magnitude and agree with the estimate[3] of 10^{12} G, despite the wide variation of B values. Consideration of other geometrical factors could increase the computed values of B_0 further and hence improve the comparison.

The ratio $\beta = n\varkappa T/(B^2/8\pi)$ is computed in column 3 and the plasmas are well under the control of the magnetic fields.

Our calculations also give the momentum of a beam electron in the cases of the fast pulsars (column 4) or the ratio $(n_b/n)(mc/p)$ for the slow pulsars (column 5). This indicates the existence of a beam of relativistic electrons with energy $\sim 10^{11}$ eV and $(n_b/n) \sim 10^{-2}$.

Relativistic electrons passing through a turbulent plasma are scattered by the turbulent fluctuations of the electric field and acquire velocities perpendicular to the magnetic field. The directions of the turbulent electric fields are confined within the cone of equation (6) so the pitch angles of the resulting spiral motion of the scattered electrons should also be limited within the same cone. Each relativistic electron, being accelerated by the magnetic field and the turbulent electric fields, then emits optical and X-ray radiation along its own direction of motion (the angular spread $\Delta\theta \sim 10^{-5}$ for 10^{11} eV). These must therefore have the same pulse width as the radio emission.

The characteristic difference between the fast and slow pulsars and the frequency dependence of the degrees of linear polarizations will provide tests of our model.

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Angular Size of the High Energy X-ray Source in the Crab Nebula

THIS article reports new data on the spatial structure of the source of 25 to 100 keV X-rays in the Crab nebula, obtained with a balloon-borne X-ray modulation collimator. The angular diameter of the Crab for energies of 1·2 to 12 keV has been measured previously in two rocket experiments by lunar occultation[1] and by modulation collimator[2]. Both measurements showed that the low energy X-rays come from an extended region one to two arc minutes in diameter centred on the visible nebula. Since then the radio[3] and optical[4] pulsar NP 0532 has been identified with a central star in the nebula[5].

NP 0532 emits radio and optical pulses with a distinctive double peak at a frequency of approximately 30 cycles s^{-1}. Similar pulsations in the X-ray flux[6] comprising 9 per cent of the 1·5 to 10 keV X-ray output of the Crab[7] have been detected which almost certainly come from the immediate vicinity of NP 0532. This suggests a two-component model for the Crab at low X-ray energies, consisting of a point source associated with the pulsar, surrounded by a nebula about two arc minutes across. The pulsar may also account for part of the non-pulsed flux. Present evidence cannot exclude a cluster of non-pulsed point sources for the nebular component of the source[8].

At higher photon energies[9] the pulsed X-ray component comprises 15 ± 5 per cent of the total 25 to 100 keV flux[10], but there have been no measurements of the angular diameter of the source at energies above 12 keV.

The experiment described here indicates that the source of 25 to 100 keV X-rays (including the pulsed component) in the Crab nebula has an effective angular diameter of at least 1·1 arc minutes and that the contribution of the point source to the total flux is no more than 40 per cent in that energy range. This contradicts the suggestion[11] that the Crab is actually a point source of X-rays and that its nebular appearance in the 1·2 to 12 keV range is caused by scattering of the X-rays from interstellar grains. If this were true, the apparent diameter of the source in the 25 to 100 keV range would be less than 0·25 arc minutes because of the rapid decrease with photon energy of the scattering cross-section of dust grains[12].

The X-ray telescope carried a pair of identical grid-modulation collimators with resolution angles of two arc minutes. The collimators, mounted together to a single frame, were supported by gimbals and stabilized in inertial space by rate integrating gyros programmed to rotate the collimators at a uniform angular rate of 16° h^{-1}, so that X-rays passing through the collimators were intensity modulated at a frequency of 8 cycles per minute. The depth of modulation depends on the angular divergence of the beam, being 100 per cent for a point source at infinity and decreasing to zero as the angular diameter of the source approaches the collimator resolution angle of two arc minutes. Behind each collimator the beam intensity was measured with a 280 cm^2, NaI(Tl) scintillation detector with active and passive shielding. The energy acceptance window, set by pulse height discriminators, was 25 to 100 keV.

The instrument was launched from the NCAR balloon base at Palestine, Texas, on May 10, 1969, and floated at 130,000 feet from 1126 to 1852 CDT within a few miles of latitude 31·1° N, longitude 93·0° W. The modulation collimators scanned the Crab ten times, averaging 10 min each. The average source intensity measured by detector A was 0·085 counts cm^{-2} s^{-1} which is consistent with results reported for a similar instrument[13]. Because of degraded sensitivity detector B recorded only half this intensity. Averages of the count rate over 1 s were telemetered to the ground and recorded on magnetic tape.

The total X-ray intensity measured by a detector was assumed to be the sum of (*a*) background radiation, (*b*) unmodulated source flux, and (*c*) modulated source flux. Components (*a*) and (*c*) were constant or slowly varying in time while (*c*) varied periodically at a frequency of about 8 cycles per minute with unknown amplitude and phase. The exact frequency depended on the pointing direction and was known from telemetered engineering data. Because of the high background intensity, component (*c*) was not directly observable in the data record. To infer the angular diameter of the source it is necessary to measure the magnitude of component (*c*) relative to (*b*). The discrete Fourier transform of the counting rate in each detector during each scan of the Crab was presented as a normalized power density spectrum. The normalized power density spectrum is the squared magnitude of the Fourier spectrum divided by the total X-ray count accumulated during the scan interval.

Each frequency component, Q_i, of the normalized power density spectrum is a random variable. At frequencies other than the modulation frequency, Q_i has an exponential probability distribution, $P(Q_i) = \exp(-Q_i)$. At the modulation frequency its probability function is

$$P(Q_j) = I_0(2\sqrt{Q_j V}) \exp(-Q_j - V) \qquad (1)$$

where I_0 is the zeroth order modified Bessels function of the first kind and V is a parameter describing the normalized amplitude of the modulated component of source flux. For a one-dimensional source model of uniform brightness and angular width, α, V is given by

$$V = \frac{(0{\cdot}81S)^2 T}{(B+S)} \left(\frac{\sin \pi\alpha/\alpha_0}{\pi\alpha/\alpha_0}\right)^2 \qquad (2)$$

where α_0 is the collimator resolution angle (2 arc minutes), T is the scan duration in seconds, B is the background intensity and S is the total source intensity averaged over T.

It can be shown that the expected value of Q_j is $(1+V)$ at the modulation frequency while at all other frequencies its expected value and standard deviation are both unity. The observed amplitude of Q_j is therefore expressed directly in standard deviations of the exponential distribution. A peak at the modulation frequency of amplitude $Q_j=1+(0{\cdot}81S)^2\ T/(B+S)$ represents a point source, while $Q_j \approx 1$ implies that the source diameter is equal to or greater than the collimator resolution angle. Because the scan rates and resolution angles of both collimators are identical, a modulated component of source flux would produce coincident peaks in the power density spectra of both detectors, while peaks due to random fluctuations would show no correlation between detectors. This increases the statistical significance of observed peaks.

Table 1. RESULTS OF DETECTOR A

(*a*) Scan duration (s)	(*b*) Source intensity (counts s^{-1})	(*c*) Expected peak for point source (standard deviation)	(*d*) Observed peak (s. d.)	(*e*) Lower limit on diameter (arc minutes)	(*f*) Upper limit on point source contribution (per cent)
540	3·3 ± 0·5	20 ± 7	5	0·4	90
720	3·2 ± 0·5	25 ± 8	1	1·1	41
540	4·7 ± 0·5	39 ± 8	3	1·1	60

Table 1 summarizes the three scans of detector A having the best source-to-background intensity ratios. The measured source intensity (column *b*) is the total intensity averaged over the scan interval less the background intensity, which was periodically monitored throughout the flight. The expected peak for a point source (column *c*) was calculated from equation (2) with $\alpha = 0$. The observed peak (column *d*) is the largest peak, within the uncertainty range of the modulation frequency, which coincides with a peak of any amplitude in the B detector spectrum (within the half-amplitude width of the peak). In every case the observed peaks are much smaller than expected for a point source, implying that the diameter of the X-ray source is about the same as (or larger than) the collimator resolution angle.

To interpret the results quantitatively, an upper limit was first placed on the strength of the modulated component by finding the smallest V which, with probability 0·99, would have produced a peak larger than that observed. This value of V was then interpreted in two ways. First it was used in equation (2), with the value of S in column *b* of Table 1, to find the width of an extended, uniformly bright, one dimensional source. The result is listed in column *e* of Table 1 as the lower limit on source diameter. The collimator transmission bands, which were horizontal, intersected the lines of declination at angles of +35 and −42 degrees for the second and third scans, so that the 1·1 arc minute limit is valid for two nearly orthogonal axes. For an alternate interpretation, the upper limit on V was used to place an upper limit on the possible point source contribution (including pulsed flux) to the total intensity of a two-component source model with the nebular component being two arc minutes in diameter. This is listed in column *f* as a percentage of the total 25 to 100 keV Crab flux. Point source contributions of more than about 40 per cent are therefore ruled out. This upper limit is consistent with previous observations[10] of the pulsed component of X-radiation.

No significant coincident peaks were found in any of the other scans, but in these other data the statistical significance is lower because of the lower counting rates.

All instrumental effects which could have smeared the modulated signal were systematically investigated before and after the flight and were found to be negligible. Tests during the flight indicated that all systems were operating properly, and I am confident that the instrument was not responsible for any significant attenuation of the modulated component and that the conclusions drawn from these data are valid. It is important, however, that these results be verified independently because they are relevant to our understanding of the mechanisms by which the pulsar could give rise to X-ray emission from the Crab.

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Characteristic Variation of Sco X-1

Rao, Rao and Jayanthi[1] have published results of their autocorrelation analysis of the optical photometry of Sco *X*-1, which they claim show a clear periodicity of about 3·0 h. They treated individual nights separately, finding respective periods of 4·43, 3·07, 2·04, 3·25, 3·00 and 0·96 h on six of the longer runs available. It seems that one should be able to say little about periodicities of the order of three hours when continuous coverage of at most eight hours is at hand, when the correlation coefficients are not very significant, and when the periods for only three of the six nights are close to three hours. Regardless, however, of opinions as to whether any significance should be attached to their analysis, we wish to point out that a much more profitable approach to studying the photometry of Sco *X*-1 is afforded by a simple inspection of the time variation. Suppose, for the moment, that the brightness changes of Sco *X*-1 were nearly random but that at irregular intervals a characteristic variation were

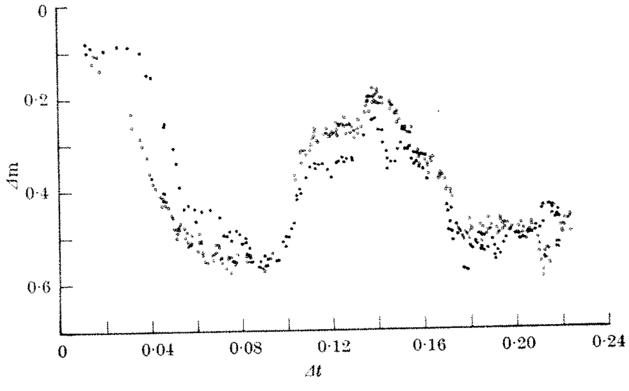

Fig. 1. Photometry of Sco *X*-1 on two nights (○, April 18, 1967; ●, May 9, 1968). The time scale is in days.

to appear for, say, a few hours. Such a variation would not be periodic and should not lead to significant periodic terms in a power spectral analysis of a long continuous sample, but it could be the signature of a physical process which, although happening at unpredictable times, behaves in a characteristic way each time that it does occur. Furthermore, in a restricted data sample, such as we have at our disposal from the published photometry, such a characteristic variation could mimic a weak periodicity without, of course, having any obligation to continue being periodic during the gaps in the observations.

To look for such a pattern, we have made graphs, on a common scale, of most of the available observations. Differences in scale factors used in graphs by different observers may be responsible for some of the obviously interesting features having gone unnoticed. In Fig. 1 we have plotted observations separated by more than a year[2,3] with the time and magnitude zero points adjusted so as to bring them into register. The adjustment in the magnitude scale is only about 0·1 magnitudes. During the first half of each run there is a feature which can be seen in whole or in part on a surprising number of nights. It looks like a distorted total eclipse, showing, in fact, all four contacts as points of sharply changing slope. We do not believe that this can actually be an eclipse because simple computations show that its duration, relative to the necessary hypothetical period, far exceeds the maximum possible duration for even a contact binary. In Fig. 2 we show photometry from a third night[2]. We have not overlaid this with the previous two graphs because visual disentangling of the three nights would begin to be difficult. The "eclipse" here is wider than those of Fig. 1, but its first stages can be overlaid very well with those of Fig. 1, as can its last stages. The general phenomenon seems to have been the same on this night, with a slight time lag between "second contact" and "third contact". Following the "eclipse" a short rise is followed by a small hill of about 0·1 magnitudes on each of the three nights. The behaviour subsequent to the hill is again similar on the three nights. Of course, as the graphs match so well, it is not only the amplitudes of these features which correspond, but also the time intervals between them. Parts of these features are seen on a number of other nights and, although one must be on guard against seeing regularities in random variations, we feel that we have been cautious and that the repetitions are unquestionably real.

We believe that we have compared all photometric observations which are useful for a study of this kind (about ten to fifteen nights, depending on the criterion for shortest useful run). We have not referenced those for which the length of the runs was too brief to show patterns on a time scale approaching six hours, but we have specifically looked at the paper by Sandage, Westphal and Kristian[4] in which some of the runs are long enough to be marginally useful. In their paper there is one interval of a few hours which follows the characteristic variation, but our main evidence is drawn from refs. 2 and 3.

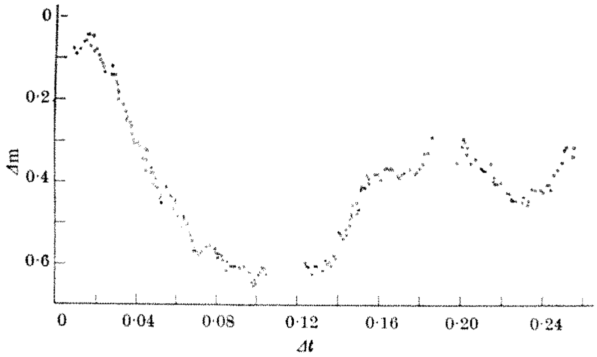

Fig. 2. Photometry of Sco X-1 on a third night (April 17, 1967). The time scale is in days.

We do not at present wish to attribute this behaviour to a particular physical process, but we find it interesting that it is not inconsistent with the mechanism recently suggested by Sofia[5] in which ionized interstellar matter is guided down magnetic field lines to be accreted onto a neutron star. That is, as the material is constrained by the field lines to follow a particular flow pattern, we may expect a consistent sequence of events whenever accretion of an interstellar cloudlet occurs.

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Water Vapour in Sunspots

Features attributable to the water vapour molecule have been detected in the spectra of several late type stars during the previous decade, particularly by Woolf *et al.*[1], who observed in the 1·13 μm, 1·4 μm, 1·9 μm and 2·7 μm absorption bands using a balloon-borne spectrometer. Spinrad and Newburn[2], Wing *et al.*[3] and Spinrad *et al.*[4] have obtained coudé and Cassegrain spectra in the neighbourhood of 0·9 μm and demonstrated the great strengthening of the water vapour absorption in this region in the spectra of many stars, more especially Mira. Sinton[5] has worked in the region 1·9 μm to 2·5 μm using a ground-based interferometer and has shown that there is water vapour absorption in the stars R Leo and χ Cyg, and McCammon *et al.*[6] have also obtained spectra in this region at Mt Wilson and Palomar. Although at the low temperatures of these stars a detectable concentration of water vapour would be expected, such measurements are of interest because they provide evidence for particular atmospheric models. The purpose of this note is to present evidence for the existence of water vapour in sunspots.

In principle, any HOH absorption band may be used for detection. Because spectra of sunspots may be obtained most easily in the visible region, however, observations of HOH lines at these wavelengths have been attempted by Wöhl[7] and by E. A. M. and D. E. B.[8]. Unfortunately the complex nature of the sunspot spectrum inhibits reliable identification here, chiefly because it is almost impossible to distinguish between enhanced telluric HOH lines and weak sunspot lines of unknown origin. In Fig. 1 we show an improved spectrum of a sunspot in the region of 5880 Å obtained with the rapid scanning photoelectric spectrometer at the Gornergrat Observatory of Oxford University[9], in Switzerland, together with the corresponding spectrum of the adjacent photosphere observed immediately afterwards as a comparison. The water vapour lines in the photospheric spectrum are due wholly to absorption in the Earth's atmosphere; their positions are indicated by arrows, and their wavelengths and identifications are taken from the second Revised Rowland Table of Moore *et al.*[10]. The dryness of the atmosphere at the Gornergrat can be judged from the shallowness of these telluric lines. This sunspot spectrum was obtained on November 18, 1969, and is of the sunspot having Rome No. 5607 which had a diameter of about 20 arc sec. The width of the spectrometer slit corresponded to $\lambda/\Delta\lambda = 1{\cdot}96 \times 10^5$. Inspection of the spectrum shows that there is indeed a small but apparently significant increase in the equivalent width of the water vapour lines, but precise measurement is uncertain because of the complexity of the sunspot spectrum. A discussion of a slightly inferior spectrum of this region has been given by E. A. M. and D. E. B.[8].

In order to increase the reliability of interpretation we have also obtained a spectrum of a sunspot in the region of 9300 Å where both photospheric and sunspot spectra are comparatively simple. For this purpose we used the Gornergrat spectrometer with an RCA infrared photomultiplier having an S1 cathode cooled with dry ice. The quantum efficiency of this type of cathode is small so that the signal is relatively noisy, but the arrangement of the Gornergrat spectrometer allows integration of the signal to be effected for an almost unlimited time with a consequent improvement in the signal-to-noise ratio. The spectrometer incorporated a Bausch and Lomb echelle grating of 100 lines mm^{-1} used with a slit width $\Delta\lambda$ corresponding to $\lambda/\Delta\lambda = 2{\cdot}0 \times 10^5$ in the sixth order, and on this occasion an integration time of 2 s per point was used. The spectrum was obtained on January 29, 1970, and is of the sunspot having Rome No. 5674 with umbral size 20 s × 10 s, on the west side of the solar disk at cos $\theta = 0{\cdot}68$. The entrance aperture of the spectrometer was 7 s × 1 s.

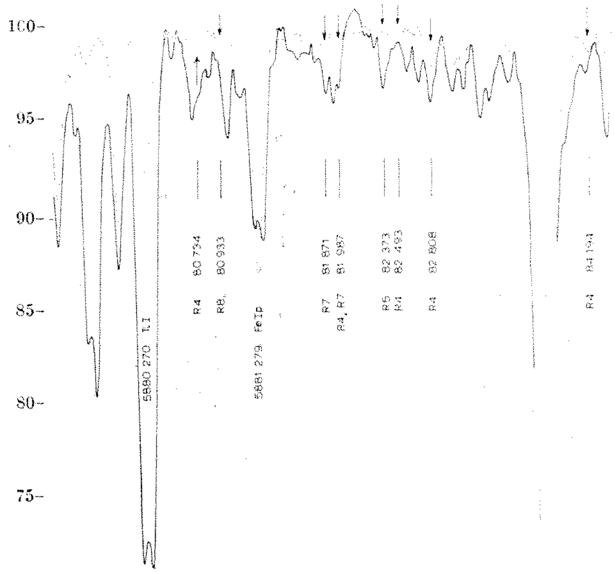

Fig. 1. Sunspot (———) and photospheric (. . .) spectra in the region of 5880 Å.

Any observed sunspot spectrum is contaminated with light scattered into the image of the spot from surrounding photospheric regions, either by the telescope or by the Earth's atmosphere. We have corrected our observed spectrum for the presence of 50 per cent photospheric light. This value was obtained by comparing the observed ratio of umbral and photospheric continuum intensities with model atmosphere calculations for a spot temperature distribution given by $\Delta\theta = 0{\cdot}3$ (where $\Delta\theta$ is the difference in θ between sunspot and photosphere, and $\theta = 5040/T$). The proportion of scattered light is high for this wavelength region, but the observations were obtained under very poor seeing conditions. The corrected spectrum, termed the "reduced" spectrum, is shown in Fig. 2, which also shows a photospheric spectrum obtained immediately afterwards using an adjacent region of the solar disk. The absorption features in this spectrum are labelled by arrows against wavelengths taken from the catalogue of Babcock and Moore[11]. These features are identified in this catalogue only as of "atmospheric origin", but a comparison with an HOH flame spectrum obtained by Spinrad and Newburn[2] at a resolving power of about 46,000 shows that the majority at least are rotational lines in the 201 vibrational band of HOH, which has a band head at 9278 Å and was first identified by Gaydon[12]. In the upper part of Fig. 3 we show smoothed versions of both the photospheric and the reduced sunspot spectra.

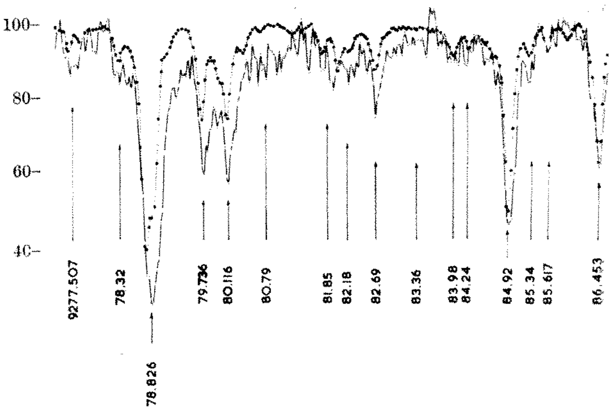

Fig. 2. Sunspot (— · —) and photospheric (● · · · ●) spectra in the region of 9300 Å.

In order to obtain the true sunspot spectrum that would be observed outside the Earth's atmosphere, termed here the "intrinsic" sunspot spectrum, we must also correct for absorption by water vapour in the Earth's atmosphere. In doing this we assume that the photospheric spectrum in this region simply delineates the telluric absorption spectrum. The result is given by the lower curve of Fig. 3. This spectrum gives the strength of the purely umbral HOH in as far as lines arising from other molecular or atomic species are absent. This analysis is complex and based on uncertain assumptions. For example, any scattering in the upper part of the Earth's atmosphere will tend to contaminate the spot spectrum with an intrinsic photospheric spectrum, and not an observed photospheric spectrum, which contains telluric lines. Further, we are obliged to assume, as is likely, that all of the photospheric absorption features are of telluric origin and treat them accordingly. These are comparatively minor matters which do not greatly affect our conclusion, however.

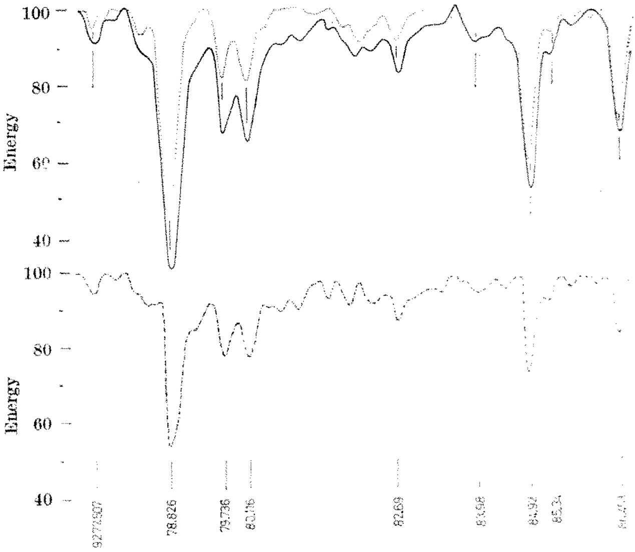

Fig. 3. Smoothed spectra of sunspot (———) and photosphere (. . .) in the region of 9300 Å, corresponding to Fig. 2. The lower spectrum (— · —) is of the spot alone as it would appear outside the Earth's atmosphere, allowance having been made for telluric water vapour lines.

Table 1. EQUIVALENT WIDTHS OF HOH LINES

Wavelength	Equivalent width (W) W_{telluric}	W_{spot}	$W_{\text{spot}}/W_{\text{telluric}}$
9278·826	189 mÅ	158 mÅ	0·84
9279·736	44	72	1·64
9280·116	59	76	1·29
9282·69	23	29	1·26
9283·98	15	12	0·83
9284·92	106	48	0·45
9286·453	68	25	0·37

It is evident from a comparison of the photospheric and reduced sunspot spectra, and from inspection of the intrinsic spot spectrum, that all of the principal HOH lines are enhanced in the spot spectrum. These lines are indicated in the upper spectra by short bars. Furthermore, the enhancement is not the same for all lines, those nearer the band head being the more enhanced. This is shown by the data of Table 1, which gives the equivalent widths of HOH lines in the photospheric (that is, telluric) and intrinsic sunspot spectra, together with the ratios of their equivalent widths in the two spectra. A detailed analysis of this water vapour band does not exist, so that an accurate assignment of J-values cannot be made. The rotational lines nearest the band head will be of high J-number, however, and we thus infer from the data of Table 1 that the J-value corresponding to maximum intensity is higher in the sunspot spectrum than in the telluric spectrum. This provides additional evidence that the extra absorption in the HOH lines originates in the spot, the shift in the position of maximum occurring because the spot has a higher temperature than the Earth's atmosphere. Although this effect is qualitatively apparent, a quantitative interpretation is not straightforward because certainly the telluric lines, and probably the stronger HOH lines in the umbral spectrum also, are saturated, or nearly so.

Some support for this interpretation of the sunspot spectrum is provided by the observed redshift of about 70 mÅ between telluric and intrinsic sunspot HOH lines. This shift is higher than that of 47 mÅ which would result from the Doppler effect arising from the rotation of the Sun, but we would ascribe such a difference to uncertainties in the reduction procedure. An increase in line width would also be expected in the intrinsic sunspot HOH lines compared with the telluric lines. The resolution of the smoothed spectrum is unfortunately insufficient to show this increase with certainty.

Another scan of the same spectral region was made on January 28, 1970, when the water vapour content of the Earth's atmosphere was considerably higher than on January 29. On this occasion the line at 9278·826 had a depth of 86 per cent in the photospheric spectrum. Even in these conditions the observed sunspot lines are clearly deeper than the photospheric lines. But although a similar analysis of this spectrum gave an intrinsic spectrum that showed HOH lines of depths similar to those in Fig. 3, the spectrum, as might have been expected, was much more noisy. This experience confirms that an extremely dry atmosphere is necessary for the detection of the amount of water vapour that we have observed in this sunspot.

The Gornergrat station of the University of Oxford is operated as part of the Hochalpine Forschungsstation Jungfraujoch. We thank the director of this organization, Dr H. Debrunner, for help with the administrative problems of the station, and the Science Research Council for a grant for erection and operation of the station.

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Determination of the Radial Velocity of a Relativistic Star

WE shall calculate the value of the radial velocity of a relativistic atom from the shift of the spectral lines by taking the transverse velocity into account. The special theory of relativity will be used.

Let us consider a system of coordinates S with rectangular axes $Oxyz$ and an atom (for example, an atom of a moving star) moving in S with a uniform velocity $\mathbf{u}$. When this atom reaches the point with coordinates $(x,0,0)$ in S $(x>0)$ it emits a photon which moves in S along the Ox axis towards the origin O where an observer is at rest. Let ν and ν_0 be the frequencies of the photon in S and in a system of coordinates in which the atom is at rest. These frequencies are connected by the relativistic Doppler formula. Following Moller[1] we have

$$\nu_0 = \nu(1+u_x/c)(1-u^2/c^2)^{-1/2} \qquad (1)$$

where c is the velocity of light and u_x the component of $\mathbf{u}$ on the Ox axis. Let $\mathbf{e}$ be the unit vector of the Ox axis (the direction of the observation), $u_x\mathbf{e}$ is the radial velocity and $\mathbf{v}=\mathbf{u}-u_x\mathbf{e}$ is the transverse velocity. Equation (1) is equivalent to

$$(u_x/c)^2(1+a^2)+2(\mu_x/c)+1-a^2+a^2(v/c)^2=0 \qquad (2)$$

where $a=\nu_0/\nu$. The two roots u_{x_i} $(i=1,2)$ of equation (2) are

$$\frac{u_{x_i}}{c} = -\frac{1}{1+a^2} - (-1)^i\,\frac{a}{1+a^2}\,[a^2-(1+a^2)(v/c)^2]^{1/2}$$

In order to obtain real numbers, the magnitude v $(0\leq v<c)$ of the transverse velocity must satisfy the relation $v\leq v_{\text{M}}$, where $v_{\text{M}}=c(1+1/a^2)^{-1/2}$. Furthermore, these roots will be satisfactory if we have $-c<u_{x_i}<c$ $(i=1,2)$. Let us remark that for a determined value of a, these roots are functions of the magnitude of the transverse velocity.

A graphical construction of these roots is represented in Fig. 1. Equation (2) between the variables v/c and u_x/c is the equation of an ellipse. Let us consider the rectangular axes associated with these variables (Fig. 1). The centre of this ellipse is at the point D $(0,-1/1+a^2)$. The values of the semi-axes DA and DB are DA$=v_{\text{M}}/c$ and DB$=(v_{\text{M}}/c)^2$. The point with coordinates $(0,-1)$ is common to all the ellipses. The values u_{x1}/c and u_{x2}/c of the roots are then respectively the second coordinates (on the u_x/c axis) of the two points M_1 and M_2 of the corresponding ellipse, the first coordinate of which is v/c $(0\leq v\leq v_{\text{M}})$. The corresponding values u_1 and u_2 of the magnitude of the velocity $\mathbf{u}$ are represented by the lengths $\text{OM}_1=u_1/c$ and $\text{OM}_2=u_2/c$. When $v=0$, only the root $u_x/c=(a^2-1)/(a^2+1)$, represented by the point B, is satisfactory; this is the solution obtained when the transverse velocity is neglected.

Two cases must be distinguished according as $a\leq 1$ or $a>1$. When $a\leq 1$, for each value of the transverse velocity such as $0<v\leq v_{\text{M}}$ the two corresponding values u_{x1} and u_{x2} of the radial velocity are negative. Thus for

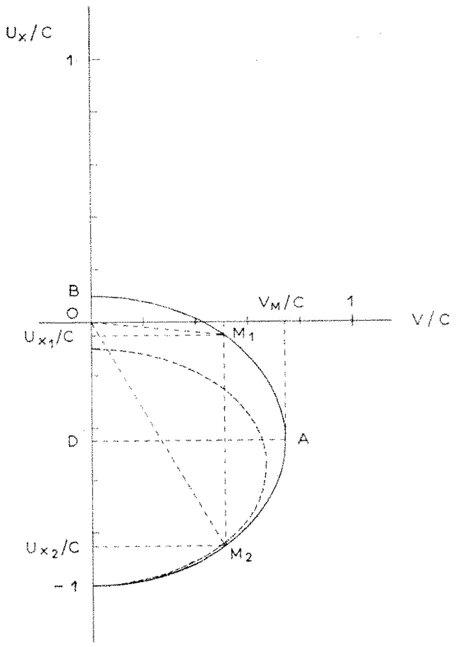

Fig. 1. Graphical determination of the radial velocity. The ellipse (———) is drawn for $a=1{\cdot}1$ (case $a>1$ in the text). For each value of v/c ($0<v\leqslant v_M$) the coordinates on the u_x/c axis of the two corresponding points M_1 and M_2 of the ellipse are the values u_{x_1}/c and u_{x_2}/c of the radial velocity. The ellipse (- - -) is drawn for $a=0{\cdot}9$ (case $a\leqslant 1$ in the text).

these values of a we may conclude that the atom moves nearer to the observer.

When $a>1$, if the value of the transverse velocity satisfies the conditions $0<v<v_0$ where $v_0=c(1-1/a^2)^{1/2}$, then the root u_{x1} is positive and the root u_{x2} is negative. Thus, if we know only the value of the ratio of the frequencies, it is impossible to state that the atom moves nearer to or away from the observer. If $v=v_0$, then $u_{x1}=0$ and u_{x2} is negative. If $v_0<v\leq v_M$, then the two roots are negative; therefore in this case we are allowed to state that the atom moves nearer to the observer.

The preceding conclusions can be applied to the case of the spectra emitted by the atoms of moving stars. So, if we know only the Doppler shift of a moving star we are not allowed to calculate the value of its radial velocity, except when the star is not a relativistic one ($u\ll c$) or when its transverse velocity is negligible compared with the radial velocity. Thus, if we consider the redshift of the spectral lines of extragalactic nebulae (corresponding to the case $a>1$), then this redshift alone cannot be a proof of the expansion of the universe.

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Reaction of Carbon and Sulphur Isotopes in Apollo 11 Samples with Solar Hydrogen Atoms

Kaplan and Smith[1] have observed that the lunar fines are significantly enriched in the heavier isotopes of carbon (^{13}C) and sulphur (^{34}S) with respect to the same isotopes in fine-grained basaltic rocks of the Apollo 11 landing site in the Mare Tranquillitatis. As a consequence they suggested that this effect may be caused by the removal of lighter isotopes (^{12}C and ^{32}S) from the unshielded lunar surface by the action of the solar wind. The mechanism of removal is thought to involve the conversion of ^{12}C and ^{32}S into $^{12}CH_4$ and $H_2{}^{32}S$, which are subsequently lost from the Moon. On the other hand, it has been pointed out at the Lunar Science Conference for Apollo 11 (January 5–8, 1970, Houston, Texas) that the solar wind may actually deposit isotopically heavier carbon on the lunar surface which then might account for the observed enrichment in the lunar fines.

Libby[2] has proposed that the Moon appears grey and essentially colourless because the solar wind brings in hydrogen from the Sun. Accordingly, the solar wind bombards the lunar surface with a velocity of about 300 km/s or more and a density of some five hydrogen nuclei per cubic centimetre[3]. After neutralization with electrons, the equivalent of 10^8 hydrogen atoms/cm²/s with an energy of 450 eV are available for chemical reactions on the Moon's sunlit side. Such conditions amount to an extremely effective chemical reducing environment. This assessment is supported by chemical analyses[4,5] of iron oxides of Apollo 11 material which are almost completely composed of FeO and not Fe_2O_3.

The deposition of carbon on the Moon is likely to involve two independent processes. The first involves transport by the solar wind, which can be estimated by combining solar wind data with those for elemental abundances in the solar system or the universe. If the solar wind is essentially of the same composition as the outer solar atmosphere, then data derived from the Sun's gaseous envelope weigh more heavily than cosmic abundance values. Table 1 lists the abundances of carbon and hydrogen for comparison.

Table 1. ABUNDANCES OF CARBON AND HYDROGEN IN ATOMS/10^4 ATOMS SILICON

	Solar atmosphere[6]	Cosmic abundance[7]
Hydrogen	$3{\cdot}2 \times 10^8$	4×10^8
Carbon	$1{\cdot}66 \times 10^5$	$3{\cdot}5 \times 10^4$

The carbon addition to the Moon based on the hydrogen content of the solar wind and hydrogen–carbon abundances can thus be calculated to be 5×10^4 (solar abundance) or 10^4 (cosmic abundance) atoms/cm²/s. Over a period of $4{\cdot}5\times10^9$ yr such a carbon contribution amounts to 140 or 28 mg/cm². Several investigators[8-10] have conducted simulation experiments involving interaction of solar wind with solids, and they conclude that the retention of different implanted atomic species is very high. Consequently we may assume that most solar wind carbon will be retained by the lunar surface. Shoemaker *et al.*[11] have calculated the median thickness of the regolith at Tranquillity Base to be 4·1 m. Supposing complete mixing within this regolith of density 1·6, the mean concentration of solar wind carbon[12,13] would be 214 or 43 p.p.m. Further, the carbon isotopic composition of the solar wind is uncertain, so that no precise estimate of the $^{13}C/^{12}C$ ratio can yet be made.

A second process placing carbon on the lunar surface involves the addition of about 2 per cent of carbonaceous chondrite-like material to the lunar soil[14]. Such material may contain about 1 per cent of carbon, resulting in a further admixture of 200 p.p.m. The $^{13}C/^{12}C$ ratio has been measured for a number of carbonaceous chondrites and varies from −18·8 to +64·4 parts per thousand with respect to the PDB standard[15,16]. It is not certain, however, what the average $^{13}C/^{12}C$ ratio is for all carbonaceous chondrites and cometary debris in the solar system, because the number and types analysed may not be representative; yet the average meteoritic $^{13}C/^{12}C$ ratio based on available data is near +20 parts per thousand, not unlike the range of +17·2 to +20·2 parts per thousand for the lunar bulk fines measured by Kaplan and Smith.

The total carbon concentration in the fines must be the sum of both processes including the native carbon content. Lunar rocks, from which the fines must be derived, contain 11–77 p.p.m. of total carbon[1] with other investigators[17] favouring 64–70 p.p.m. This then is taken to be the indigenous carbon concentration at Mare Tranquillitatis. The total carbon concentration should therefore range from 239 to 417 p.p.m., but the measured value is in the 100–200 p.p.m. range[18-20].

The question thus arises: what has happened to the missing carbon? Apparently solar wind noble gases are not all quantitatively captured or retained, but a 50 per cent loss of carbon seems to be too great for a non-gaseous element.

Returning to the effect of solar hydrogen, as well as its reducing character the high impact velocity must be considered. This leads both to implantation and to radiation damage of minerals in exposed surfaces, foremost in the lunar fines, in which imbedded atoms penetrate to a depth[21] of some hundreds of Å. Because bonds involving ^{12}C or ^{32}S atoms are weaker than those involving ^{13}C or ^{34}S, decomposition is more likely to affect the light isotopes. As a result of lower zero point energy, more energy is required to break a heavier isotope bond than a similar bond extended from a lighter one. Consequently, isotope effects must be expected which are generally greatest for the lightest elements and which decrease with increasing temperature. The following reaction scheme is proposed with the understanding that some of the sulphur may sublime directly and account for its low presence:

Solar protons → Decomposition of minerals with attendant isotope effect and acquisition of loosened electrons by solar protons → Reaction of hydrogen with preferentially exposed ^{12}C and ^{32}S → Liberation of $^{12}CH_4$ and $H_2{}^{32}S$

The maximum ratios in specific rate constants at 300 K between different isotopes of the same chemical element have been calculated by Bigeleisen[22]. For the pair ^{12}C, ^{13}C the ratio is 1·25, and for ^{32}S, ^{34}S about 1·03 (Bigeleisen, personal communication). These values are strictly a guide of what to expect at most for the lunar chemistry of the Apollo 11 landing site. It is particularly important to emphasize that we do not know the precise chemical composition of all the carbon and sulphur compounds in this location. The lunar fines, however, provide a much larger surface area than the rock pieces examined. The fines should therefore be depleted more so in the lighter isotopes of carbon and sulphur. This difference between carbon and sulphur isotopes is indeed reflected in the analysis of the Apollo 11 samples reported by Kaplan and Smith[1]. The δ^{13}C values relative to the PDB standard of $-18·8$ to $-29·8$ parts per thousand for fine-grained rock compare with $+17·2$ to 20·2 parts per thousand for the bulk fines, a difference of some 40 parts per thousand. At the same time the δ^{34}S values relative to the Canyon Diablo standard range from $+1·2$ to 1·3 parts per thousand for solid rock pieces, and from $+4·4$ to 8·2 parts per thousand for fines corresponding to a difference of some 4 parts per thousand. The δ-values for carbon and sulphur thus show a net difference of one magnitude. This is in good agreement with theoretical expectations based on maximum differences in isotopic reaction rates. Moreover, similar data obtained by the same authors on breccias are intermediate in value between those for fines and rocks. Measurements by Epstein and Taylor[23] on lunar fines and breccia are also in agreement with this work. Furthermore, it is unlikely that the isotopic differences are caused by terrestrial contamination, because the fines most likely to be affected (as a result of their large surface area) contain the heavier carbon.

The ultimate fate of the newly formed gas molecules is their escape into the almost negligible lunar atmosphere. As shown previously, the chance of a gas molecule leaving the lunar surface with a speed v in excess of the escape velocity is $1 - V_E/V$ where V_E is the lunar escape velocity of 2·38 km/s. The effective time of escape of gases in an atmosphere as a function of atomic weight has been discussed by Jastrow and Rasool[24]. Consequently the lifetime of undissociated $^{12}CH_4$ near the lunar surface can be calculated to be approximately 100 yr, while that for undissociated $H_2{}^{32}S$ is of the order of the age of the solar system. But no H_2S atmosphere has been observed on the Moon, so the effective atmospheric residence time must be considerably less. This is, most likely, because of the destructive effects of photo dissociation and the sweeping action of the solar wind. In contrast, methane and hydrogen sulphide trapped inside rocks are likely to last much longer even though methane, being a smaller molecule, will be lost more quickly by diffusion processes. Indeed, Epstein and Taylor have reported the liberation of H_2S after heating lunar rocks and detected trace quantities of methane[23].

On balance there seem to occur on the Moon several processes capable of producing a heavier isotopic composition of carbon and sulphur in finely divided soil relative to solid rock. The total proton bombardment of the Moon since the formation of the solar system is equivalent to $10^8 \times 3·15 \times 10^7 \times 4·5 \times 10^9 = 1·4 \times 10^{25}$ atoms/cm^2 equivalent to about 23 moles of hydrogen/cm^2 averaged over the entire lunar surface. It is difficult to see how such an excess coupled with considerable kinetic energy would not react with carbon or sulphur. A test of this hypothesis would be to measure the carbon content and isotopic composition of fines in the polar regions of the Moon and to compare it with that from the sunlit areas. If carbon from the poles were isotopically lighter as opposed to the Sun-exposed region, then solar wind erosion would indeed be proven.

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Daily Variation of the Geomagnetic Field at the Dip Equator

THE range ΔH_A of the daily variation of the horizontal component of the geomagnetic field at Alibag (A), a low latitude station outside the influence of the equatorial electrojet, is largely caused by weakening of the ambient field on the night side rather than an enhancement on the day side[1-3]. The effect may be caused by magnetospheric drift currents which, being more than 30,000 km above the surface of the Earth, would produce almost identical effects at Alibag and Trivandrum (T), situated on the dip equator 1,100 km south of Alibag, if differences in the induced field arising from local ground conditions are ignored. On the other hand, the well known enhancement of ΔH at the dip equator is caused by the influence of the equatorial electrojet. Consequently $\Delta H_T - H_A$ must be related to purely ionospheric currents.

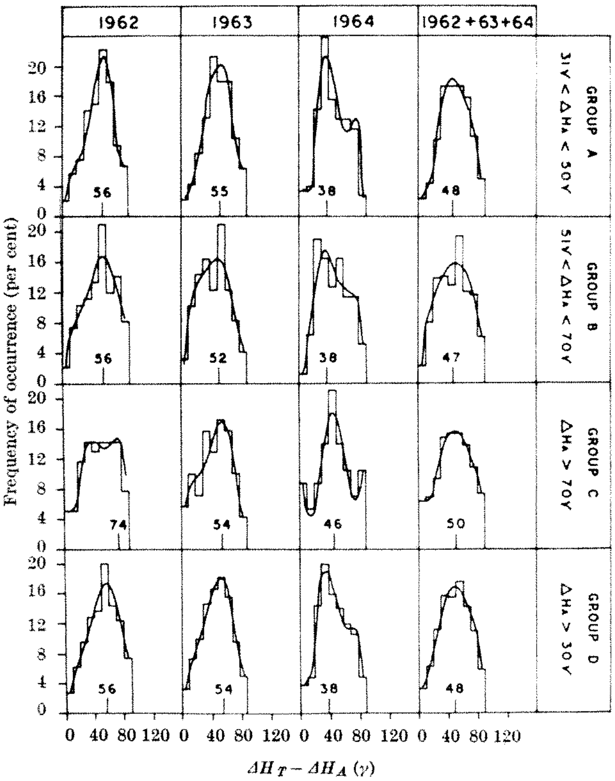

Fig. 1. The normalized histograms of (ΔH_T–ΔH_A) for different ranges of ΔH_A for the years 1962, 1963, 1964 and for all the three years combined.

We examine here the validity of these assumptions particularly because they contradict the dynamo theory, which suggests that the contribution of ionospheric currents should predominate at both stations on days which are not geomagnetically disturbed. We also estimate the relative contributions of the two processes at Trivandrum and at Alibag.

Fig. 1 shows the normalized histograms of $\Delta H_T - \Delta H_A$ for different ranges of ΔH_A for the years 1962, 1963, 1964 and for all the three years combined. On a day-to-day basis, ΔH_A is more or less independent of the purely ionospheric contribution $\Delta H_T - \Delta H_A$. Similarly, as shown in Table 1, ΔH_A is independent of the changes of F region ionospheric electron density related to the midday mean value of f_0F_2 over Kodaikanal, which is also close to the dip equator. Thus ΔH_A does not track the changes in the ionosphere.

Since January 1964, regular measurements of the electron drifts in the E and F regions of the ionosphere have been made at Thumba, near Trivandrum, by recording the fading of radio reflexions at three spaced aerials. The drift was always to the west during the day. The mean drift speed between 1100 and 1300 h correlates well with $\Delta H_T - \Delta H_A$ shown in Fig. 2. Rocketborne magnetometers have measured the magnetic field of the electrojet current in the E region[4]. R. Raghava Rao (private communication) has shown, using current density derived from similar measurements and simultaneously observed electron density, that the average electron velocity is comparable with the drift velocities measured by the spaced aerial technique. A linear relationship between the drift speed in the E region and $\Delta H_T - \Delta H_A$ is therefore consistent with the interpretation that the drift velocity measured by the spaced aerial technique is proportional to the electron drift velocity in the electro-jet and that moreover the latter, at noon, can be deduced from $\Delta H_T - \Delta H_A$, which is derived exclusively from ground measurements.

Table 1

ΔH_A range	Mean ΔH_T-ΔH_A (γ)	Mean midday f_0F_2 (MHz)	Number of days
31 to 50 γ	50	7·3	319
51 to 70 γ	51	7·6	314
71 γ and above	49	7·7	292
31 γ and above	50	7·6	925

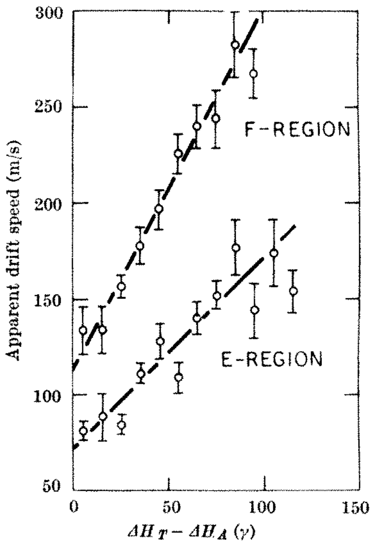

Fig. 2. The relation showing the mean westward apparent drift speed during midday hours (1100–1300 h) for different values of ΔH_T–ΔH_A.

Electron and ion currents in the F region would produce equal and oppositely directed magnetic effects, and therefore the correlation of $\Delta H_T - \Delta H_A$ with F region electron drifts indicates that the latter are themselves correlated with the E region drifts. Comparing the magnetograms from Tamale (dip 1° 14′) and Legon (dip 10° 11′), Osborne[5] has argued that the electrojet cannot be a simple enhancement of the normal current in a belt of high conductivity. Moreover, Osborne and Skinner[6] have also shown a correlation between the electrojet strength and the F region drifts. The conclusions of these authors are therefore consistent with ours.

According to the dynamo theory, the mean current density $J = \overline{\Sigma}' \cdot E$, where $\overline{\Sigma}'$ is the height integrated conductivity and E is the primary electric field. The derived values of $\overline{\Sigma}'$ for E region at 0°, 30°, 60° and 90° dip latitudes[7] enable a smooth curve to be drawn to express the variation of conductivity with latitudes. For this, the ratio of conductivities at Trivandrum and Alibag is derived as

$$\frac{7\cdot 58\times 10^{-8}}{2\cdot 61\times 10^{-8}}=2\cdot 9$$

Table 1 shows that the average ionospheric contribution at Trivandrum is about 50γ. Consequently, the average ionospheric contribution to ΔH_A would be $50/2{\cdot}9 \approx 17\gamma$.

The monthly mean values of $\Delta H_T - \Delta H_A$ and ΔH_A are shown in Fig. 3 separately for the years 1961, 1962, 1963 and 1964, and for the four years all together, along with the midday mean values of f_0F_2 at Kodaikanal, which is also close to the dip equator. $\Delta H_T - \Delta H_A$, which is purely a result of ionospheric currents, shows clear equinoctial maxima. Drifts at Thumba also exhibit equinoctial maxima[8]. In contrast with the purely ionospheric effect, ΔH_A has no consistent seasonal variation.

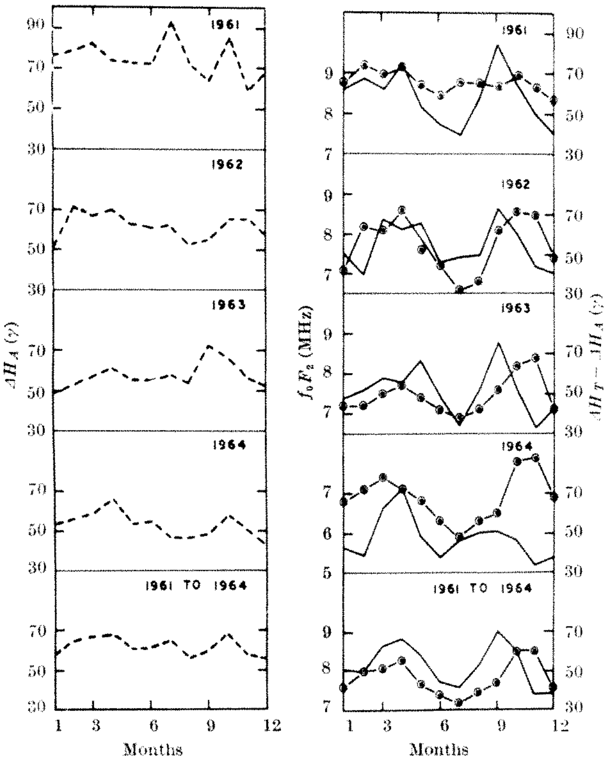

Fig. 3. The monthly mean values of ΔH_A, $\Delta H_T - \Delta H_A$ and f_0F_2 at Kodaikanal for the years 1961, 1962, 1963, 1964 and for all the four years combined. - - - -, ΔH_A; ——— $\Delta H_T - \Delta H_A$; ●—●, f_0F_2.

According to Hones and Bergeson[9], a radial electric field generated by the wobbling of the Earth's magnetic axis in the magnetosphere, distorted by the solar wind, should cause charged particles to experience net energy changes over a number of revolutions around the Earth. From this theory, an explanation for the enhancement of the equatorial electrojet at equinoxes can be visualized. Assuming that the kinetic energy density of the solar wind, which compresses the magnetosphere in the sub-solar direction, is constant over a period of one year, the Earth's magnetic axis is more nearly perpendicular to the solar wind flow at equinoxes than at solstices. The compression of the magnetosphere and the radial electric field produced in the ionosphere is therefore a maximum during equinoctial months. This effect should be more pronounced at the equator than at higher latitudes.

We conclude that the contribution to ΔH_T at Trivandrum, which can unambiguously be attributed to an ionospheric current, remains largely unaffected by the magnetospheric contribution at both Trivandrum and Alibag. This supports the contention that two independent processes are operating. While at Trivandrum the ionospheric contribution is similar in magnitude to the magnetospheric contribution on a normal day, at Alibag the ionospheric contribution is only about a third of the magnetospheric effect. Moreover, our results support the view that drift velocities measured by the technique of spaced aerials are related to the velocity of electrons in the electrojet.

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"Two-way Stretch" of Sialic Crust and Plate Tectonics in the South-west Pacific

Analyses of seismic and continuous gravity measurements over the northern Tasman Sea[1] have confirmed the existence of "quasi-continental" crust (20–26 km thick) beneath the parallel ridges—Norfolk Ridge, Lord Howe Rise and Dampier Ridge—that extend north-westward from New Zealand (Fig. 1). The intervening major trough, New Caledonia Basin, is shown to have a floor of true oceanic thickness (9 km).

The recognition of a crustal thickness of 20 km along Dampier Ridge is particularly important, for it effectively invalidates van der Linden's[2] interpretation of this morphological feature as an extinct or dormant mid-ocean ridge. Apart from seismic activity and high heat-flow values, mid-ocean ridges are distinguished[3,4] by crustal thicknesses less (usually much less) than those of the normal ocean floor. It is inconceivable that a relatively thick crustal segment like Dampier Ridge could have operated as a centre of sea-floor spreading during Cainozoic or late Mesozoic times.

Also inconsistent with evolution as a mid-ocean ridge are the asymmetric location of Dampier Ridge within the narrow northern neck of the Tasman Basin and its abrupt termination at the point where the latter broadens and where sea-floor spreading between Australia and New Zealand has obviously been at a maximum. Moreover, detailed examination of the local bathymetry reveals that the southern end, at least, of Dampier Ridge is linked with Lord Howe Rise at depths greater than 3,000 m. Dampier Ridge clearly represents a segment of sialic crust, structurally and genetically analogous to Lord Howe Rise and Norfolk Ridge. Because of negative seismic[5] and heat-flow[6] data from the north Tasman Sea, van der Linden's interpretation is based solely on morphology and an apparent symmetry of low amplitude geomagnetic anomalies across a narrow section of Dampier Ridge. It is not shown that the geomagnetic pattern of Dampier Ridge is significantly more symmetrical than that of Lord Howe Rise[7] or Norfolk Ridge[8]; neither is the possibility considered that other mechanisms might participate in the development of geomagnetic anomaly patterns.

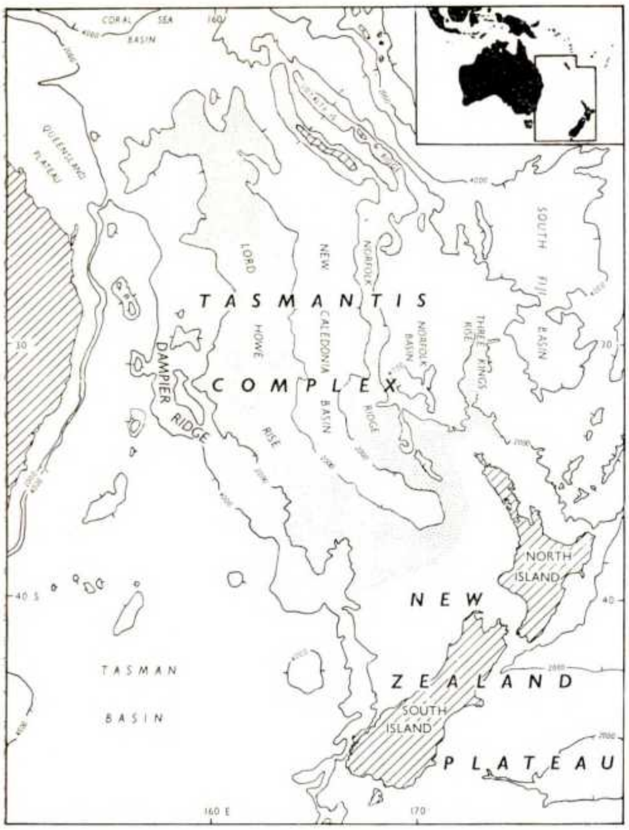

Fig. 1. Sketch-map of the submarine morphology of the Tasman Sea, showing the main ridges (stippled) and basins of the Tasmantis Complex. Landmasses shaded. Only the 2,000 m and 4,000 m isobaths are shown.

An alternative explanation of the structural relationships of the parallel ridges and basins north-west of New Zealand (collectively termed the "Tasmantis Complex"[9]) is proposed here. Expressed simply, the ridges of the Tasmantis Complex are regarded as segments of sialic crust of intermediate thickness, separated from the eastern margin of the Australian craton and rifted apart—in a manner comparable with the spreading of fingers of a hand—by mantle convection currents migrating in a general north-eastward direction from the Indian–Antarctic mid-ocean ridge, south of Australia[10,11]. The intervening long, narrow basins that developed during this process are presumably floored, as in the case of New Caledonia Basin[1], by newly formed crust of oceanic or near-oceanic thickness. Concomitant widespread extrusion of basaltic lavas on the flanks, and even the crest, of the resultant ridges is envisaged as producing crudely symmetrical geomagnetic anomaly patterns, reminiscent of mid-ocean ridges.

The Tasmantis Complex structure has been further complicated by differential northward displacement of Australia with respect to the New Zealand crustal segment. Since the disruption of Gondwanaland, northward migration of Australia has greatly exceeded that of the New Zealand Plateau, although the geomagnetic chronology derived for the south-west Pacific by Heirtzler *et al.*[12] demonstrates that the separation of Australia from Antarctica postdated that of New Zealand by some 40 m.y.

The Tasmantis Complex, spanning the gap between Australia and the New Zealand Plateau and formerly continuous with the Queensland Plateau and New Guinea[9,10,13], has consequently been subjected to intense stress. This is manifested by the sharp northward swing in strike of its component ridges and basins as the Australian coast is approached, by the development of multiple NW–SE fractures traversing the ridges in response to northward extension, and by the wrenching apart, on either side of the Coral Sea Basin, of the New Guinea and Tasmantis ridges, with the abrupt termination of the latter at latitude 18°–19° S.

These effects were accompanied by the attenuation of Norfolk Ridge and the northward extension and eventual disruption of yet another ridge farther east, separated remnants of which are now represented by Loyalty Islands Ridge and Three Kings Rise. The latter is here regarded as a sialic segment comparable with the other Tasmantis ridges and not, as van der Linden[2] claims, a possible second extinct mid-ocean ridge. Interpretation of Three Kings Rise and Dampier Ridge as extinct mid-ocean ridges is also excluded by the complete absence of any evidence of crustal compression such as trench formation in the region between the two ridges. The available data suggest that the region is in fact dominated by N–S and E–W tension.

The parallelism of the Tasmantis ridges and basins can probably be attributed, in part at least, to regional structural control by a system of Palaeozoic–Mesozoic geosynclinal and geanticlinal belts along the eastern periphery of the former Gondwana supercontinent. An intermediate stage in the evolution of the Tasmantis Complex may, however, have involved the development of parallel north–south megashear zones resulting from the differential northward migration of Australia. As suggested by Standard[14], the seamount chains of the north Tasman Sea probably represent such major shear zones in the thin oceanic crust. The formation of the alternating sialic ridges and oceanic basins of the Tasmantis Complex can be explained by subsequent expansion of the belts of crustal weakness, thus created, by north-eastward spreading mantle convection currents emanating from the Indian–Antarctic Ridge. The eastward limit of these currents, which are still active, is marked by the seismic Kermadec, Tonga and New Hebrides island arc and trench system.

The mechanism described here provides an explanation of the known structural and morphological features, the crustal thicknesses, and the seismic and heat-flow zonations in the south-west Pacific. It is considered preferable to van der Linden's assumption of extinct mid-ocean ridges at Dampier Ridge and Three Kings Rise, which is neither consistent with recent data nor helpful in clarifying the evolution of the very active island arc–trench system of the south-west Pacific and its associated Benioff zone[15]. In that they deal with the interrelationships of adjoining segments of sialic and oceanic crust in a critical sector of the Earth's surface, and raise the possibility of spreading of marginal sialic crust as well as of purely oceanic crust, the opinions stated above have implications for the concepts of sea-floor spreading and plate tectonics.

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Magnetized Basement Outcrops on the South-east Greenland Continental Shelf

Most continental shelves are included in the "Pacific" and "Atlantic" categories[1]. The Pacific type marks lines along which lithospheric plates underthrust or move past one another along strike slip faults. It is not surprising, therefore, that such shelves are characterized by irregular relief, vulcanism, faulting and great seismicity. The Atlantic type represents edges of continents that were once contiguous but have been rifted apart by plate movements, with new oceanic crust created between the rifted edges by sea-floor spreading. Most shelves of the Atlantic type are depositional. A wedge, thickening towards the sea, of largely undeformed Cretaceous and younger sediments along the eastern United States attests to gradual subsidence.

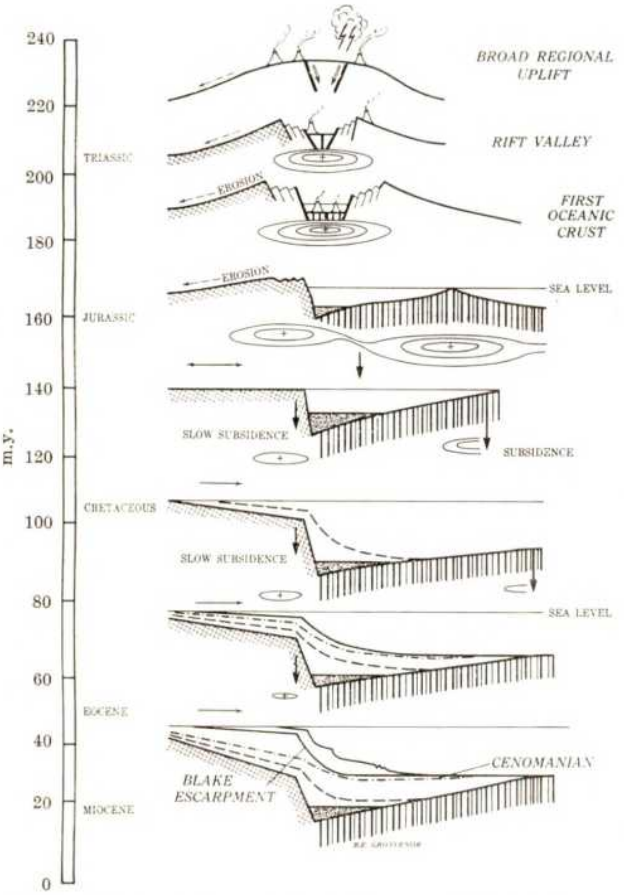

Fig. 1. Evolution of rifted continental margin in central North Atlantic. The continental basement subsides by erosion, and the disappearance of the thermal anomaly is inherited from the initiation of rifting. Note that the basement reaches sea level 50 to 100 m.y. after the ocean basin forms. Further subsidence is accelerated by sediment and water loading. Based on refs. 2 and 3.

Ostenso and I[2] have suggested that the initial margins of the continents, at first warped upwards, are in time reduced to sea level by erosion and thermal contraction (about 100 m.y. after the beginning of rifting in the central North Atlantic). Further subsidence may reflect sediment loading near the edge of the shelf and continued subsidence because of thermal contraction by cooling. The hypothesized evolution of the North American continental margin, based on the above premises, is shown in Fig. 1 (taken from Schneider[3]).

If this hypothesis is correct, it is reasonable to expect to observe margins in various stages of development at the present time. The East African rift is represented by the first two depicted stages, and the Red Sea by the third.

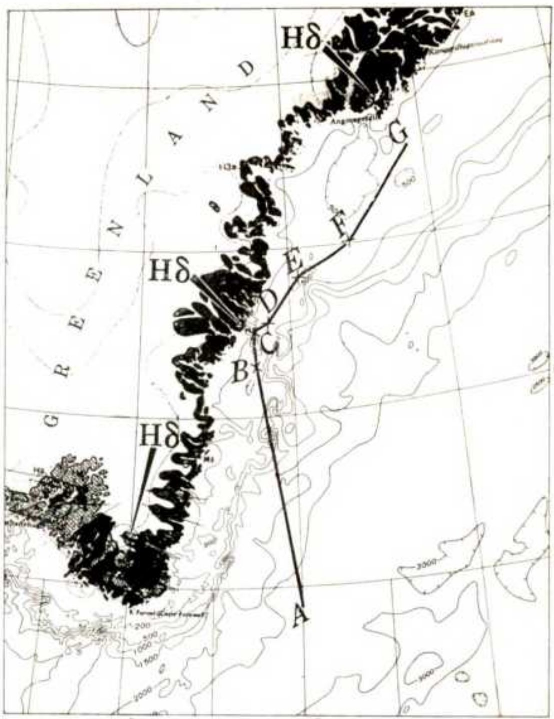

Fig. 2. Location of bathymetric–magnetic traverse (*A–G*) across south-east Greenland shelf, and adjacent coastal geology[6]. Black denotes Archean gneisses and migmatite, reworked by Hudsonian orogeny; Hδ denotes post-orogenic granitic intrusives.

So far most margins of the Atlantic resemble the last three stages of Fig. 1, with igneous or metamorphic basement complexes usually deeply buried under the seaward reaches of continental shelves.

A bathymetric–magnetic traverse across the south-east Greenland shelf (Fig. 2) seems to reveal the fourth or fifth evolutionary stage of Fig. 1. The regions of rough, short-wavelength topography and magnetic anomalies suggest where basement is probably exposed (Fig. 3). An eroded basement surface has been lowered to sea-level but is not yet buried by a wedge of sediments younger than the rift that separated Scandinavia and the British Isles from Greenland. The age of the oldest ocean floor between Greenland and Rockall–Hatton Bank has now been confidently dated[4] at 60 m.y. BP.

We should thus expect the margins of the Norwegian Sea to be going through the stage that the north central Atlantic had achieved by Jurassic times, that is, after the oceanic basin was already of the order of 60 m.y. old (Fig. 1).

The bathymetric–magnetic traverse (Figs. 2 and 3) also yields some conclusions more specifically related to Greenland. The sea-floor topography of the shelf consists of broad depressions (probably glacial valleys carved when the sea level was lower[5]) with widths of about 50 km and depths about 400 m below the average shelf levels. These features also occur farther north along the Greenland coast and in other glaciated areas[5].

There are two varieties of shelf micro-topography—rough and smooth (Fig. 3). The rough type exhibits amplitudes of 50 to 100 m and wavelengths of a few km or less. Near *E* there is rough small-scale topography within a broad valley. Most probably these undulations are glacially abraded, igneous and metamorphic shield rocks similar to the predominant Archean gneisses and migmatites, reworked by the Hudsonian orogeny[6], that are exposed on the nearby mainland (Fig. 2). The vigorous East Greenland current scours the shelf and may help to keep sediments from burying the topography. The rough basement is associated with magnetic anomalies of similar wavelength, with amplitudes up to several

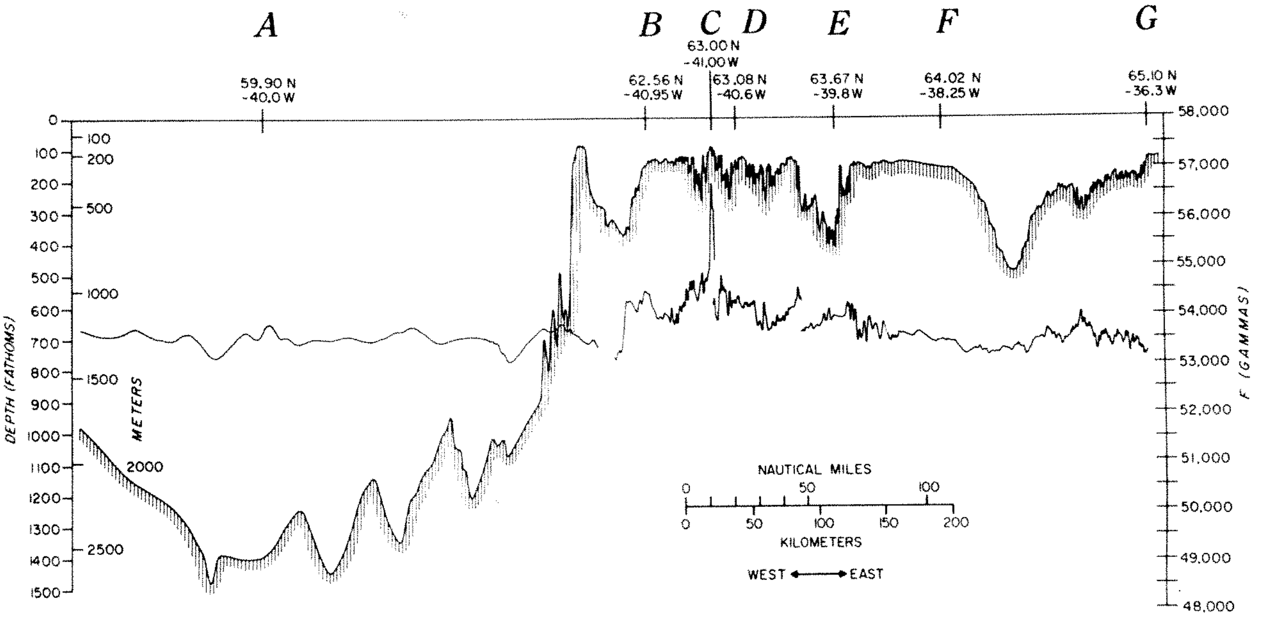

Fig. 3. Bathymetric (shaded) and total magnetic field profile across south-east Greenland shelf. Position of track shown in Fig. 2 (*A*–*G*).

hundred gammas. A sea-floor high near the coast at *C* is associated with a conspicuous 2,800 gamma anomaly.

The smooth micro-topography is in general associated with a smooth magnetic field, suggesting relatively uniformly magnetized or weakly magnetized metamorphic and sedimentary formations. Possibly the magnetized basement has been downfaulted and covered by younger sediments. To some degree the correlation between anomalous magnetic field and topography may imply the existence of a rather uniform highly magnetized (basaltic ?) layer, with the anomalies resulting from relief characteristics on this surface. The Keweenawan basalts in northern Wisconsin exemplify this case[7]. In the present example, however, the magnetically disturbed shelf areas occur offshore from granitic intrusions, and complex high-amplitude anomalies are likely to occur in the vicinity of such intrusions because of a complex and highly variable magnetization[8]. The granitic intrusions on the Greenland coast are reported to be Sanerutian or younger. Some may even be lower Tertiary in age, by analogy with the dated (60 m.y. BP) Rockall granite[9,10], which was very near the Greenland intrusions about 60 m.y. BP[4], and which also exhibits a circular region of high amplitude, short-wavelength magnetic anomalies around its centre[11].

Another explanation for the short-wavelength magnetic and bathymetric features is that they reflect dike swarms, probably early Tertiary in age, similar to those observed along coastal Greenland in the Tertiary basalt province[12]. The density of dikes (10 to 100 per mile[12]), their high magnetization, and their probable resistance to erosion make them an attractive explanation, although there is no other evidence of Tertiary dike swarms so far south of the basalt province.

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"Pan African Orogeny" in the Coastal Region of Ghana

SEVERAL authors[1-4] have advocated that in West Africa the 500–600 m.y. "Pan African Orogeny"[5] was a complete orogenic cycle and not merely a thermal event that caused isotopic changes. It has been postulated that in Ghana[3,4] this cycle began when the Birrimian rocks, which had been involved in a previous orogeny between 1,700 and 2,000 m.y. ago, became the basement to a sedimentary cover sequence comprising the Togo (Akwapimian, Atacorian) and Buem Formations and lower parts of the Voltaian Group (Fig. 1) which are in part laterally equivalent to one another and in which there is a south-eastward transition from miogeosynclinal to eugeosynclinal facies. The cover and basement to the south-east of a line running approximately between Accra and Niamey (inset of Fig. 1) were then involved in the thermo-tectonic phase of the "Pan African Orogeny" while that to the north-west remained essentially undisturbed as part of the West African craton. Within the orogenic zone the cover was strongly folded and thrust on south-easterly dipping planes, was lightly metamorphosed, and perhaps locally subjected to granitization[6,7] and to granite intrusion, while the Birrimian basement was reactivated to give the unit known as the Dahomeyan.

One essential part of this hypothesis is that in the orogenic zone the Birrimian was reactivated (that is, the rocks were presumably deformed and metamorphosed again) at the same time as the cover sequence was being folded, thrust and metamorphosed for the first time. This has yet to be substantiated by a detailed analysis of the thermo-tectonic evolution of the rocks involved.

The coastal strip of Ghana is well suited for such an analysis, being a relatively well exposed cross-section from the Birrimian of the West African craton in the west and centre, through the Togo Formation in the southern extension of the Atacora–Togo–Akwapim Range in which the formation is supposed to have been effected by the orogeny, to the Dahomeyan (reactivated Birrimian) in the east. A programme of research is in progress in this coastal region to establish the tectonic and metamorphic evolution of the Togo Formation which will then represent the effects of the "Pan African Orogeny" on the cover in the region. A second aim is to recognize these effects on the Dahomeyan. In this way it is hoped that the tectonic and metamorphic history of the Dahomeyan before the "Pan African Orogeny" can be determined and then compared with that of the Birrimian in the craton; according to recent interpretations these histories are closely similar. So far only the Togo Formation and Dahomeyan have been studied in any detail, but already results are available which are not in agreement with current opinion and cast doubt on the concept of a complete orogenic cycle having taken place in this region 500–600 m.y. ago. A full account of these two units is in preparation and, in the meantime, the principal points and conclusions are outlined here.

The sequence of deformational and metamorphic events in the Togo Formation and Dahomeyan is summarized in Table 1, where a correlation of events is also made between the two units. It is thus clear that the Dahomeyan had suffered at least two phases of intense deformation (DF_1 and DF_2) before the deposition of the Togo Formation and that the earlier phase was accompanied by moderate to high grade metamorphism. The regional schistosity in the Dahomeyan was produced during the earlier phase, while the flat-lying attitude of the schistosity was the result of the recumbent folding of this fabric during the second phase. In contrast to previous ideas, therefore, neither the regional Dahomeyan schistosity nor its attitude is related to any phase of deformation which affected the Togo Formation, and so neither can be in any way attributed to the "Pan African Orogeny".

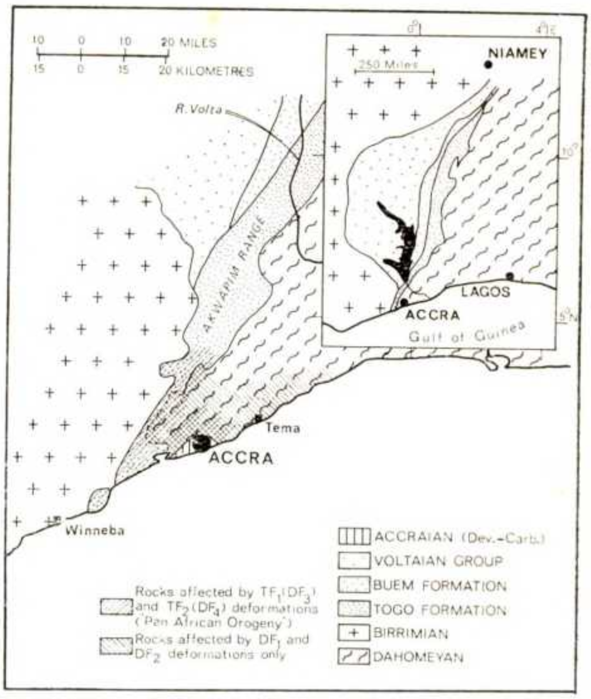

Fig. 1. Regional distribution of the Voltaian Group, Buem and Togo Formations, Birrimian and Dahomeyan in central West Africa (inset) and in the coastal region of Ghana.

There is also evidence in south-east Ghana to oppose the idea that the deformation associated with the orogeny increases in intensity from north-west to south-east from the craton into the orogenic zone. On the contrary, there is no indication that the Dahomeyan rocks in the coastal region were deformed during the two tectonic episodes which affect the Togo Formation (TF_1 and TF_2) except in the area immediately adjacent to the outcrop of the formation in the Akwapim Ridge and in windows through the formation (Fig. 2). Moreover, while these two tectonic phases exerted a strong influence on the morphology of the region by producing the north-east–south-west topographic grain in the prominent Akwapim Ridge, they were very mild from a deformational point of view, giving rise to only small strains and only local penetrative tectonic fabrics. Further, the low greenschist facies metamorphism which accompanied the first phase is related more to deformational processes than to a regional metamorphism resulting from a general temperature rise during an orogeny.

In the light of these results it seems that the concept of the 500–600 m.y. event involving a complete orogenic cycle in Ghana at least needs to be reconsidered in the light of detailed tectonic and metamorphic evidence obtained from the rocks involved. I do not question here that the geosynclinal stages of an orogenic cycle are manifested in the cover sequence, but I do contest that two minor phases of deformation, which are not accom-

Table 1. SEQUENCE OF DEFORMATIONAL AND METAMORPHIC EVENTS

	Dahomeyan	Togo Formation
"Pan African Orogeny"	DF_4. (Immediately adjacent to and in windows through the Togo Formation of the Akwapim Ridge.) Upright fold of DS, schistosity and DS_2 boudinage on NNW/SSE axial planes, hinges plunging gently N. Monoclinal flexures, open small-scale folds, kink bands and crenulation cleavage. Possibly major open Nungua Anticline related. Insignificant metamorphism.	TF_2. (Local development.) Upright folds of bedding and TS_1 slaty cleavage on NNW/SSE axial planes. Typically gentle flexure folds but local chevron folds and kink-bands. Insignificant metamorphism.
	DF_3. (Immediately adjacent to and in windows through the Togo Formation in the Akwapim Ridge.) NE/SW striking axial planes dipping mainly to SE at moderate angles but local conjugate set dipping NW. Typically open monoclinal flexures of DS_1 schistosity but set with SE dipping axial planes locally tight to isoclinal with rare DS_3 axial plane slaty or crenulation cleavage. Insignificant metamorphism.	TF_1. (Widespread.) Axial planes strike mainly NE/SW and dip SE at moderate angles but considerable variation. Plunge mainly NE in Akwapim Ridge but variable in the coastal section proper. Typically gentle flexural folds of bedding but locally tight with TS_1 axial plane slaty cleavage in SE flank of Akwapim Ridge. Syntectonic very low greenschist facies metamorphism.
	DF_2. (Widespread development.) Axial planes strike mainly NW/SE, dipping gently to NE; hinges plunge mainly to N at low angle but subsidiary maximum plunge E/W. Recumbent tight to isoclinal folding of DS_1 and bedding. Contemporaneous boudinage of bedding and DS_1 fabric on axes plunging mainly N at low angles. Very local development of DS_2 axial plane slaty or crenulation cleavage. No significant metamorphism.	
	DF_1. (Widespread development.) Original axial plane orientation unknown because of subsequent deformation—now flat-lying. Hinges plunge mainly to NW or N at low angles. Isoclinal folds of bedding with strong regional DS_1 axial plane schistosity and B-lination. Syn- and latectonic metamorphism in epidote amphibolite and granulite facies.	

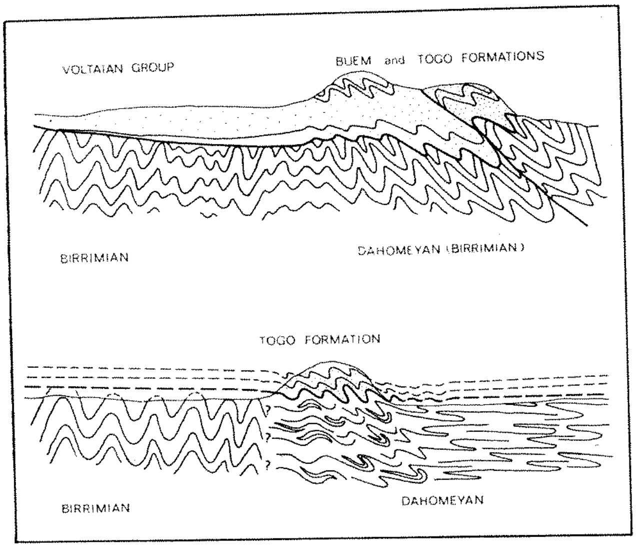

Fig. 2. Diagrammatic NW to SE sections from Birrimian of the West African craton overlain by undisturbed cover to deformed cover and Dahomeyan. Upper section through central Ghana as interpreted by Grant[4], and lower section through the coastal region of Ghana as interpreted here.

panied by any significant metamorphism and do not affect the basement more than locally, should be given the status of a full tectonothermal phase in an orogenic cycle. It is therefore impossible to accept the idea that the Dahomeyan rocks are those of the Birrimian which were deformed and metamorphosed again to a degree sufficient to give them their present very different aspects.

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Pattern of Relative Motion from Fracture Zone and Spreading Rate Data in the North-eastern Pacific

THE concepts of sea floor spreading and plate tectonics provide a useful framework within which to examine the tectonic history of oceans and continents[1–4]. In these new theories, the Earth is envisaged as a complex mosaic of rigid plates in constant interaction and the instantaneous relative motion between any two of these plates can be represented by a rotation about a pole. Morgan[3] pointed out that sections of fracture zones should follow small circles centred about such poles. This generalization is useful in deriving the path of continents which have been separated by continental drift, for the fracture zones mark the lines of movement.

Although, to a first approximation, continental drift can be described by a small number of finite rotations about different poles, it has not been demonstrated that such a simplified model is a valid representation of reality. The largest (and unique) portion of the ocean floor where the bathymetry has been mapped in extraordinary detail and the pattern of magnetic anomalies has been well recognized is the north-eastern Pacific (ref. 5 and unpublished work of H. W. M. with T. C. Chase). This portion of the ocean floor, which comprised two plates, is thus an excellent area in which to analyse the pattern of relative motion between two large plates.

At least five different directions have been recognized for the major fracture zones in the north-eastern Pacific (ref. 6 and unpublished work of H. W. M. with T. C. Chase). When these fracture zones are aligned according to the younger of the two anomalies on either side, the changes of direction pointed out by H. W. M. and Chase (unpublished) align surprisingly well (Fig. 1). This strongly suggests that these changes of direction, being contemporaneous, reflect true changes of relative motion between the north-east Pacific and Farallon plates[7]. Fracture zones of correlatable trend lie between magnetic anomalies 10–16 (phase 1), 16–25 (phase 2) and 25–32 (phase 3). Two other phases (phases 4 and 5) can be distinguished on portions of the ocean floor older than magnetic anomaly 32.

A pole of relative rotation that best fits the orientation of the fracture zones for each phase was calculated. This pole minimizes, in the least squares sense, the angular distance between the pole and the points in each fracture zone. It was determined by a search method and corresponds to the minimum value of the pooled estimate of the standard deviation of the angular distances. The 95 per

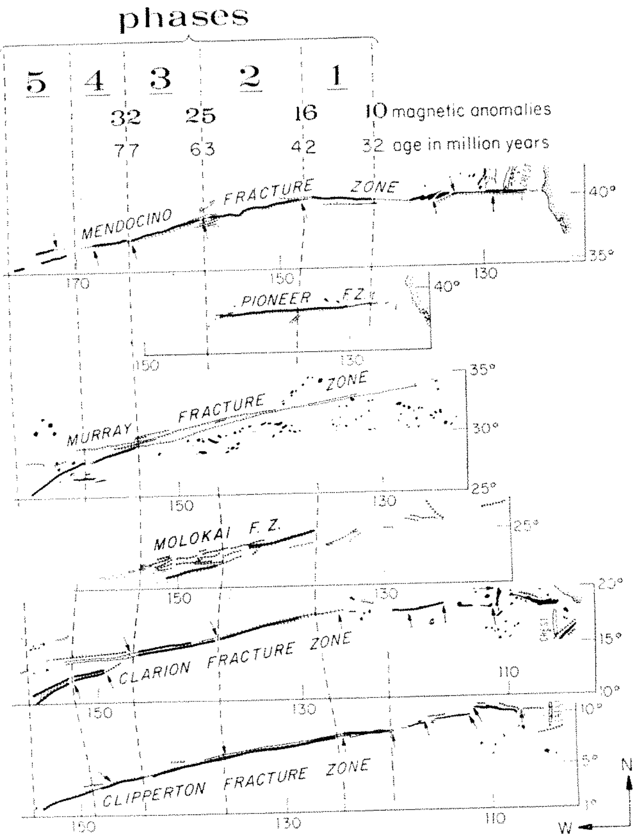

Fig. 1. Fracture zones in the north-eastern Pacific aligned by the younger of the two anomalies on either side of the fracture zone. The arrows are the changes of direction of H. W. M. and Chase (unpublished). The heavy black line represents the sections of the fracture zones used to compute the poles. The Murray fracture zone has not been considered for phases 1 and 2. During this time interval it behaved as a leaky transform fault and there was a jump in the spreading centre below the Murray[8].

cent confidence limit on the minimum standard deviation enables an oval of confidence to be drawn around the best fit pole. The computed ovals of confidence are large and elongate (Fig. 2). The poor estimate of the small curvature results in a large major axis perpendicular to the direction of the fracture zones. In contrast, the control in the position of the poles of relative motion in a direction parallel to the fracture zones is good. The ovals do not intersect and so the poles of relative motion are significantly different from one another with the exception of phase 2 and phase 3 where the fracture zone data are ambiguous.

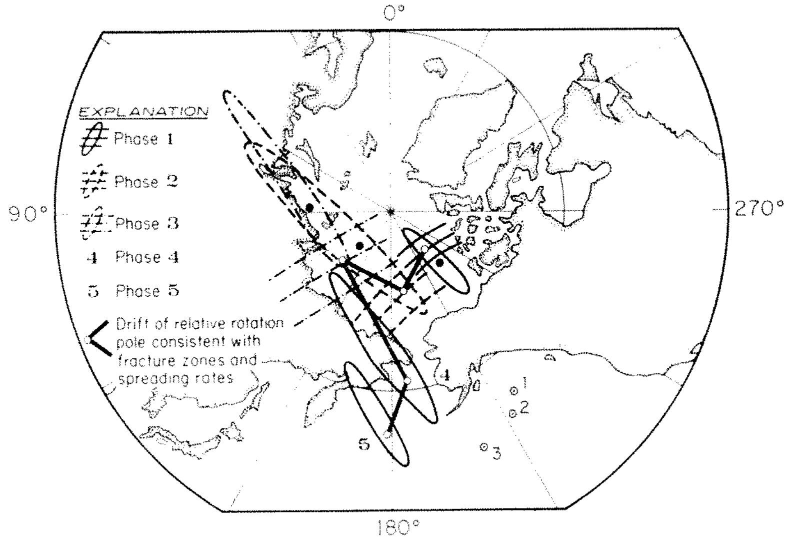
0°
EXPLANATION
Phase 1
Phase 2
Phase 3
4 Phase 4
5 Phase 5
Drift of relative rotation pole consistent with fracture zones and spreading rates
90°
270°
1
2
3
4
5
180°

Fig. 2. Poles of relative rotation north-east Pacific/Farallon. Mean and 95 per cent band of confidence for the angular distance of the pole of relative rotation north-east Pacific/Farallon are determined from the spreading rates for magnetic anomaly intervals 8–13, 13–21, 23–32. Also shown are the pole (black dot) and oval of confidence for the fracture zone data for magnetic anomaly intervals 10–16, 16–25, 25–32. The heavy line joining white dots represents the drift of the relative pole north-east Pacific/Farallon consistent mean with both fracture zone and spreading rate data. The points 1, 2, 3 are the positions of localities used to compute mean angular distance of pole from spreading rate data for phases 1, 2 and 3.

Because the fracture zone oval gives reasonable control on the direction on which the pole of relative rotation has to lie, it is possible to compute from spreading rate determinations the distance of a given point of spreading from this pole. Seven mean spreading rates have been measured[5] between magnetic anomalies 8–13, 13–21 and 23–32. The anomalies were selected because they are prominent and the time spans they embrace overlap sufficiently the time intervals for which the fracture zones keep a constant trend. Spreading rates are proportional to the sine of the angular distance between the pole of relative motion and the localities where spreading is measured. The distance to the pole can thus be estimated by fitting a sine curve to the measured spreading rates. The information from spreading rates provides a good control in the direction perpendicular to the fracture zones. We have estimated statistically a band at the 95 per cent level about the mean angular distance from a point of the plate to the pole after all measurements have been adjusted to this point. The estimated bands for the pole of relative motion for phases 1, 2 and 3 intersect the respective ovals of confidence obtained from fracture zone data (Fig. 2). This combined method improves greatly the control in the position of the poles (Table 1). For example, the pole positions for phases 2 and 3 are now significantly different. The poles of the relative motion, north-east Pacific/Farallon, follow a distinct path and move at least 30 degrees during the time interval covered by the north Pacific fracture zones.

Table 1. COMPARISON BETWEEN RELATIVE POLE POSITIONS NORTH PACIFIC/FARALLON DETERMINED FROM SPREADING RATES AND FRACTURE ZONES

	Fracture zones		Spreading rates and fracture zones	
Phase	Latitude	Longitude	Latitude	Longitude
1	78° N	224° E	81° N	224° E
2	82° N	138° E	77° N	185° E
3	76° N	88° E	78° N	132° E
4	61° N	185° E		
5	52° N	178° E		

Several conclusions can be drawn from this analysis. (1) Even in an area where the ocean floor has been extensively mapped, the knowledge of fracture zone trends is not adequate to specify a reliable position for a pole of relative motion (Table 1). This is largely a result of the shortness of the segments of fracture zones where the trend remains approximately constant. This point is demonstrated most clearly in Figs. 14 *a–e* of ref. 3. Where the further information of spreading rates is available the control in the pole position is greatly improved. This result casts doubt on the feasibility of tracing reliably the direction of continental drift from fracture zone surveys in the "quiet" magnetic zones of the Pacific and Atlantic Oceans. (2) The generalization that present poles of relative motion tend to cluster around the Earth's geographic pole does not seem to be true for the past motion of the north-east Pacific and Farallon plates. This is because the path of the poles of relative motion and the palaeomagnetic polar path are both fixed in a frame attached to the north-east Pacific plate and these two paths lie on opposite sides of the North Pole[8]. (3) It seems plausible *a priori* to postulate that large original offsets of the ridge crests (that is, long transform faults) could stabilize the relative motion of two plates. In the north Pacific, even though major offsets in the ridge crest were present, the movement of the north-east Pacific/Farallon poles suggests that these transform faults did not control the basic motion of either plate over a time interval of 50 to 100 m.y. (4) Because plate movements are interrelated, any major change in the pattern of spreading must be global[4]. It is therefore possible that the sharp bends in the path of the pole, north-east Pacific/Farallon, might be correlated with contemporaneous changes of the relative motion of any two other plates. In particular it would be interesting to find out whether changes in the direction of Atlantic and Indian Ocean fracture zones occur at anomaly time 10, 16, 25 and 32, and whether these changes are related to particular collisions of continents[9]. (5) The changes in the relative motion of the north-east Pacific and Farallon plates have been frequent—one every 10 to 20 m.y. The physical significance of such changes is not very clear because the driving mechanism of the plates has not been elucidated.

The north-east Pacific fracture zone and spreading rate data are consistent and indicate that rigid plate tectonics may be a valid model to use in attempts to reconstruct the past positions of continents. One further implication of the movement of the pole of relative rotation is worth stressing. It has been shown that three plates cannot rotate through finite angles about a single set of three axes of relative rotation fixed to the respective pairs of plates[4,7]. The pole of relative rotation, north-east Pacific/Farallon, has, however, not remained fixed to these two plates. If this behaviour is typical of all other plates (and recent Atlantic data suggest that this might be so: refs. 10, 11 and personal communication from B. C. Heezen), it shows that none of the relative rotations need keep constant parameters and all three rotations may adjust to each other with time[4]. This will introduce great complexity in attempting palaeogeometry. On the other hand, this extra degree of freedom may make it possible to apply large displacements to the plates of the Earth and still retain the simple boundaries of the plate theory defined by Wilson[12], McKenzie and Parker[2] and Morgan[3].

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Highly Undersaturated Rocks in Upper Mantle Conditions

OLIVINE nephelinite and the closely related magmas, basanite and olivine melilite nephelinite, are products of widely distributed but small volcanic eruptions. They commonly contain dense, coarse grained xenoliths produced at high pressures in the upper mantle, particularly lherzolites, pyroxenites and garnet pyroxenites. These magmas therefore derive directly from the mantle and cannot have been modified by shallow, crustal fractionation processes[9]. It is important to know the variation of the mineralogy of olivine nephelinite as a function of pressure and temperature. Exploratory measurements using a piston-cylinder high pressure apparatus to 36 kilobars, and using techniques which have given equilibrium mineral assemblages in basaltic composition[1], have shown a variation in the mineralogy of a nepheline-rich basic composition at sub-solidus temperatures over the pressure range of the crust and upper mantle. The composition studied (Table 1) has very low albite and is classified as transitional between olivine nephelinite and nepheline-rich basanite. The results of the experiments are given in Table 2; phases were identified by X-ray powder diffraction and by optical examination. What follows is a discussion of the mineral reactions required by the data of Table 2.

Table 1. COMPOSITION OF THE SYNTHETIC OLIVINE NEPHELINITE GLASS USED IN THE EXPERIMENTAL WORK

		CIPW norm	
SiO_2	44·3	Or	6·1
TiO_2	1·5	Ab	2·0
Al_2O_3	14·2	Ne	15·3
Fe_2O_3	0·5	An	19·4
FeO	9·7	Di	26·4
MnO	0·2	Ol	25·9
MgO	13·3	Ap	1·3
CaO	11·2	Il	2·9
Na_2O	3·6	Mt	0·7
K_2O	1·0		
P_2O_5	0·5		
$\frac{100\ Mg}{Mg + Fe^{2+}}$	71·0		

The absence of detectable nepheline (amounting to less than 10 per cent) in the low pressure assemblages (4·5 kilobars, 9 kilobars), and the presence of plagioclase and spinel, can be accounted for by the following reactions, in which substitution of Fe for Mg is neglected:

$$\underset{\text{diopside}}{mCaMgSi_2O_6} + \underset{\text{anorthite}}{(n+2)CaAl_2Si_2O_8} + \underset{\text{nepheline}}{NaAlSiO_4} \rightleftharpoons \underset{\text{aluminous diopside}}{mCaMgSi_2O_6.2CaAl_2SiO_6} + \underset{\text{plagioclase}}{nCaAl_2Si_2O_8.NaAlSi_3O_8} \tag{1}$$

$$\underset{\text{anorthite}}{3CaAl_2Si_2O_8} + \underset{\text{olivine}}{2Mg_2SiO_4} + \underset{\text{nepheline}}{NaAlSiO_4} \rightleftharpoons \underset{\text{diopside}}{2CaMgSi_2O_6} + \underset{\text{spinel}}{MgAl_2O_4} + \underset{\text{plagioclase}}{CaAl_2Si_2O_8.NaAlSi_3O_8} \tag{2}$$

These reactions will limit the occurrence of nepheline in olivine-bearing plutonic rocks to very shallow depths in the Earth's crust and illustrate the equivalence of clinopyroxene + plagioclase + spinel ± olivine assemblages to olivine nephelinite.

The elimination of plagioclase between 9 kilobars and 13·5 kilobars is due to the reaction between olivine and plagioclase[2] to yield aluminous clinopyroxene solid solution and spinel. Orthopyroxene cannot be detected and calculation of the pyroxene + spinel + olivine mineralogy suggests that the clinopyroxene at intermediate pressures is largely diopside + jadeite + (Ca,Mg)–Tschermak's silicate solid solution. In the pressure interval of 13·5 kilobars to 25 kilobars the mineral assemblage is clinopyroxene + spinel + olivine with olivine decreasing in abundance in favour of spinel at higher pressure. Olivine is not detectable in the 22·5 kilobar and 24·8 kilobar runs but a mica (possibly phlogopite) is present in small quantities. The decrease in olivine and increase in spinel are attributed to the reaction of olivine with Ca–Tschermak's silicate (see equation (1)) in the clinopyroxene solid solution

$$\underset{\text{(diopside + Ca–Tschermak's silicate)}}{mCaMgSi_2O_6.CaAl_2SiO_6} + \underset{\text{olivine}}{Mg_2SiO_4} \rightleftharpoons \underset{\text{diopside}}{(m+1)CaMgSi_2O_6} + \underset{\text{spinel}}{MgAl_2O_4} \tag{3}$$

Garnet is first observed in the 27 kilobar run and garnet and olivine increase at the expense of clinopyroxene and spinel with increasing pressure. The idealized reaction (reaction (4), neglecting pyroxene solid solution) yields grossular-rich garnet. Breakdown of any Mg–Tschermak's silicate solid solution in the pyroxene may contribute a higher pyrope content to the garnet (reaction (5))

$$\underset{\text{diopside}}{2CaMgSi_2O_6} + \underset{\text{spinel}}{MgAl_2O_4} \rightleftharpoons \underset{\text{olivine}}{Mg_2SiO_4} + \underset{\text{pyrope-grossular}}{Ca_2MgAl_2Si_3O_{12}} \tag{4}$$

$$\underset{\text{(diopside + Mg–Tschermak's silicate)}}{mCaMgSi_2O_6.MgAl_2SiO_6} \rightleftharpoons \underset{\text{grossular-pyrope}}{CaMg_2Al_2Si_3O_{12}} + \underset{\text{diopside}}{(m-1)CaMgSi_2O_6} \tag{5}$$

It may be noted that the pressure required for the appearance of grossular-rich garnet in this composition closely matches that obtained for the appearance of grossular-rich garnet in the simple system (diopside + spinel[3]) and for a

Table 2. MINERAL ASSEMBLAGES FORMED BY REACTION OF OLIVINE NEPHELINITE COMPOSITION AT 1,100° C AT VARIOUS PRESSURES

Pressure (kilobars)	Temperature (° C)	Time (h)	Phases present	Comments
1 atmosphere	1,100	4	Ol + Cpx + Pl + glass	
1 atmosphere	1,000	24	Ol + Cpx + Pl	Minor plagioclase
4·5	1,100	2	Ol + Cpx + Pl + Sp + ? glass	Minor spinel. Possible incipient melting
9·0	1,100	4	Ol + Cpx + Pl + Sp + ? glass	Less olivine than at 4·5 kilobars Possible incipient melting
13·5	1,100	4	Ol + Cpx + Sp	Minor olivine, spinel increased from 9 kilobars
18·0	1,100	4	Ol + Cpx + Sp	Rare olivine, spinel increased from 13·5 kilobars
22·5	1,100	4	Cpx + Sp + mica	Olivine not detected, minor mica
24·8	1,100	4	Cpx + Sp + mica	
27·0	1,100	4	Cpx + Sp + Ga + mica	Large euhedral garnet
36·0	1,100	4	Ol + Cpx + Sp + Ga + mica	Minor olivine and spinel, rare mica

Experimental conditions are given in the first column; the mineralogy produced is listed in the fourth column.
Ol, Olivine; Cpx, clinopyroxene; Ga, garnet; Sp, spinel.

marked increase in grossular content of garnet in aluminous basaltic compositions (kyanite eclogite, grosspydite)[4]. At very high pressures, elimination of spinel from the assemblage may yield grossular-rich garnet + olivine + (omphacite). Hydrogen was not excluded from the sample and this allowed the formation of mica at pressures above 18 kilobars. Our data indicate that mica (phlogopite) may occur in the upper mantle in olivine-rich compositions at pressures in excess of 30 kbars and temperatures up to at least 1,100° C (ref. 10).

Comparison of the data with previous data in basaltic compositions[1,4] shows that, in the olivine nephelinite composition, plagioclase disappears at a lower pressure and garnet requires a very much higher pressure to form. Assemblages of intermediate and high pressure granulite facies in the olivine nephelinite composition do not consist of garnet + aluminous pyroxene + plagioclase mineralogy as is characteristic of most classes of basaltic rocks[1], but instead of clinopyroxene + plagioclase + spinel ± olivine and clinopyroxene + spinel ± olivine. The very high pressure required to form garnet contrasts with the behaviour of basaltic and peridotitic compositions and points to the stability of clinopyroxene + spinel ± olivine mineralogies in very undersaturated nephelinitic compositions in the conditions of high temperature eclogite facies (> 20 kilobars at 1,100° C). Yoder and Tilley[11] reported clinopyroxene + spinel + mica assemblages in olivine nephelinite at 31·4 kilobars and 40 kilobars, 1,200° C. The composition used (containing normative larnite) is very much more undersaturated and has a higher Fe_2O_3 content than the present composition. The differences in high pressure mineralogy are attributed to these differences in composition.

The mineral assemblages obtained in our experiments are comparable with chemically similar natural rocks (although with lower Na_2O content) crystallized as aluminous clinopyroxene + spinel + olivine and occurring as xenoliths in basanitic magma[5]. Some of these xenoliths contain garnet, but the garnet is pyrope–almandine and not grossular-rich. These xenoliths imply crystallization in the 10–20 kilobar pressure range[6,7].

The grosspydite inclusions[8] in Russian kimberlite pipes contain grossular-rich garnets and probably require pressures similar to the garnet-bearing assemblages of the olivine nephelinite. These rocks differ chemically from the olivine nephelinite in higher Al_2O_3 and lower MgO and Na_2O contents, so that intermediate pressure assemblages are of garnet + plagioclase + pyroxene and the high pressure assemblages contain kyanite rather than olivine and spinel[4,8].

In considering the origin of olivine nephelinite magmas in an upper mantle of peridotite composition, it is apparent that the mineral components of the source rock which may enter a melt to produce liquid olivine nephelinite will be mainly aluminous clinopyroxene + spinel + olivine at depths to around 100 km and may be omphacitic clinopyroxene + olivine + grossular-rich garnet ± spinel at greater depths. The evaluation of the possible mechanisms of production of olivine nephelinite liquids requires direct melting studies, but it is important that orthopyroxene and pyrope–almandine components should be enriched in the residue rather than the liquid in melting of a garnet peridotite source[9].

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High-magnesian Calcite and Protodolomite in Lake Balaton (Hungary) Sediments

RECENT sedimentation in Lake Balaton is governed chiefly by two processes[1]. (*a*) Clastic material (quartz, feldspars, calcite, dolomite, phyllosilicates, heavy minerals) introduced by wind and rivers is distributed on the bed by the currents. (*b*) During the warmer seasons much fine-grained high-magnesian calcite is precipitated from the lake (average depth, 3·3 m; July average temperature, 25° C) because of the dense and extensive growth of phytoplankton. In photosynthesis CO_2 is liberated from dissolved bicarbonate, and carbonate is precipitated.

The precipitates are equant grains or aggregates of irregular shape and have rounded surfaces. About half the grains (chiefly aggregates) are 2–6·3 μm in size, and the rest smaller. The precipitates $MgCO_3$ concentrations (Fig. 1*a*) are between 6 and 8·5 mole per cent. The concentrations show a clear tendency to increase from west to east along the axis of the basin. The increasing incorporation of $MgCO_3$ into the calcite lattice (determined by X-ray methods) runs more or less parallel with an increase in the total $MgCO_3$ content of all carbonates present in the 2–6·3 μm grain class of the sediments. The

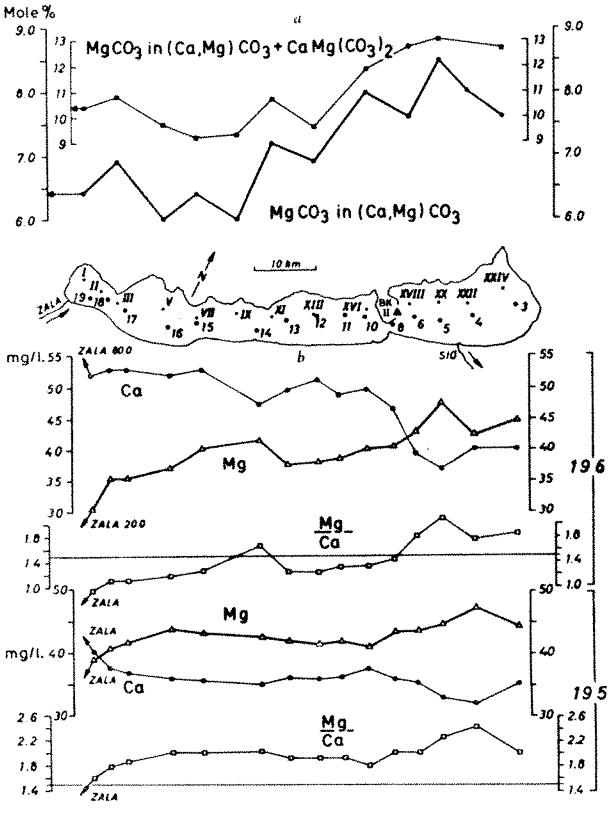

Fig. 1. *a*, $MgCO_3$ contents (in mole per cent) of high-magnesian calcites and total $MgCO_3$ concentrations of carbonates in the grain size class 2–6·3 μm (carbonates = 100 per cent) in Recent sediments of Lake Balaton (sampling stations I–XXV); *b*, Ca and Mg contents of Lake Balaton water and Mg/Ca ratios during two periods of investigation (sampling stations marked 3–19), after ref. 4. The coring station is marked "BK II".

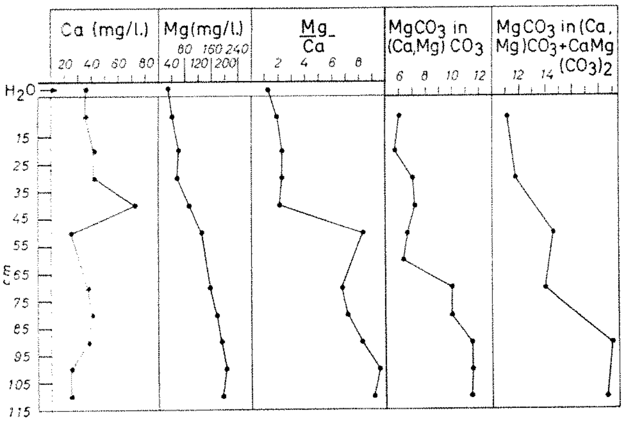

Fig. 2. Ca and Mg contents and Mg/Ca ratios of interstitial waters in sediment core "BK II" and $MgCO_3$ contents (in mole per cent) of high magnesian calcites and total carbonates in the 2–6·3 μm grain class.

greater values found (by chemical methods) come from the presence of small amounts of dolomite as well as the high-magnesian calcite derived from precipitation.

Table 1. AVERAGE COMPOSITION OF LAKE BALATON TRIBUTARIES AND LAKE BALATON WATER, AFTER REF. 2

	Inflowing water mg/l.	Lake Balaton mg/l.
K^+	5·6	6·1
Na^+	18·1	27·0
Ca^{2+}	80·6	32·0
Mg^{2+}	36·1	47·8
HCO_3^-	336·8	280·0
Cl^-	9·0	12·0
SO_4^{2-}	46·1	55·0

Recent formation of high-magnesian calcite in a fresh water environment—which to my knowledge has not yet been reported elsewhere—can be attributed here to the very large Mg/Ca ratio in comparison with other fresh water environments. It is clear from Table 1 that the average composition of the lake water differs markedly from that of the water coming to the lake (chiefly its westernmost basin) from rivers and springs. The lake water is relatively enriched in K^+, Na^+, Mg^{2+}, Cl^- and SO_4^{2-} and depleted in HCO_3^- and Ca^{2+}.

Two factors are responsible for this change in hydrochemistry. (*a*) The rate of evaporation in the lake area is higher than the rate of precipitation. According to Szesztay[3], the amount of water that leaves Lake Balaton via the Sió-canal in the eastern part of the lake is more than 25 per cent smaller than the inflow from the hinterland

$$C + H = P + L$$
$$630\text{ mm} + 860\text{ mm} = 870\text{ mm} + 620\text{ mm}$$

where C is the average annual precipitation, H the average annual inflow from the drainage area, P the average annual evaporation and L the average annual outflow via the Sió-canal. Increasing concentrations from west to east thus result. (*b*) Because of the precipitation of high-magnesian calcite caused by the photosynthesis of the phytoplankton, large amounts of Ca^{2+} and HCO_3^- (plus minor amounts of Mg^{2+}) are extracted from the lake water.

Both factors combined lead to the observed enrichment of most of the ions but to a depletion of Ca^{2+} and HCO_3^- in the lake. Fig. 1*b* shows the Ca and Mg concentrations (in mg/l.) and the Mg/Ca ratios[4] of the lake water on June 28–29, 1960, and June 23–25, 1959. From the mouth of the Zala (western part) to the eastern part of the lake the Ca^{2+} concentrations decrease but the Mg^{2+} concentrations and Mg/Ca ratios increase considerably. For the 1960 investigation the Ca and Mg curves intersect in the eastern part of the lake, whereas for 1959 the intersection point is only a few kilometres from the mouth of the Zala River. These differences are caused by annual climatic changes. The greater the inflow of river water and the amount of precipitation, the smaller are the rates of evaporation and photosynthesis and the further east is the intersection point, and vice versa. When the surface of the lake is covered by ice and photosynthesis is reduced to a minimum, there might be no intersection at all.

The general increase of the Mg/Ca ratio in the water of Lake Balaton from west to east corresponds to the observed increase of the $MgCO_3$ concentration in the precipitated high-magnesian calcite.

A 1·15 m sediment core taken near the Tihany peninsula (Fig. 1) was also studied. The results of the X-ray and chemical analyses of the sediments and interstitial waters are shown in Fig. 2.

In the interstitial waters the Mg concentrations increase with depth and the Mg/Ca ratio reaches values (9·5) that are greater than in seawater. The increase of the Mg/Ca ratio corresponds to an increase of the $MgCO_3$ incorporated in the high-magnesian calcite, which in the deepest parts of the core shows values of 11·5 mole per cent. My explanation is that the water of Lake Balaton has considerably changed its chemistry during the past thousands of years. The very large Mg/Ca ratios found in the deeper part of the core might indicate the period before the lake was first regulated by the Romans, during which an outflow existed only occasionally. This could explain the generally greater concentrations of the lake water (other ions than Mg are also enriched). The Ca content of the water, however, decreased because of the precipitation of high-magnesian calcite.

Note added in proof. In addition to the clastic dolomite (stoichiometric dolomite), which occurs mainly in the coarse silt, and sand fractions of all sediments, authigenic protodolomite (Ca_{56-58} Mg_{44-42}) has been detected in the clay fraction of most of the bottom muds. This finding resembles in many ways the formation of protodolomite in the muds of the Neusiedler Sea (Austria/Hungary)[5] in an environment with a high Mg/Ca ratio.

The Biological Research Institute of the Hungarian Academy of Sciences at Tihany kindly provided facilities for sampling.

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Direct Lattice Resolution of Layer Planes in Polyacrylonitrile Based Carbon Fibres

THE microstructure of polyacrylonitrile (PAN) based carbon fibres has been investigated by several techniques including high and low angle X-ray diffraction and electron microscopy. The results obtained with high-modulus (400 GNm^{-2}) RAE carbon fibres[1,2] and high-modulus Rolls-Royce carbon fibres[3] are essentially similar; crystallites, the widths of which have a distribution of values with a mean about 65 Å, enclose sharp-edged microvoids of mean width about 10 Å. There is little three-dimensional order in the crystallites and the graphite layer planes are essentially turbostratic. The length of the crystallite seems to be limited by lattice defects to less than 100 Å although individual layers may well be much longer. Studies of rayon-based carbon fibres[4] have yielded similar evidence and recently Fourdeux, Hérinckx, Perret and Ruland[5] reported that the layer planes in these materials can be resolved directly by high-resolution electron microscopy following the phase-contrast method described by Heidenreich, Hess and Ban[6] for carbon black specimens.

Allen, Cooper and Mayer[7] have discussed the preparation of carbon fibres of exceptionally high modulus (545 GNm^{-2}) after the incorporation of boron by annealing at high temperature. One of their samples which contained a nominal 1 per cent boron after annealing at 3,000° C, and a sample annealed at 2,500° C without boron, were made available for microstructural investigation. Experience with Rolls-Royce carbon fibres suggests that microstructural differences between carbon fibres of similar strength annealed at temperatures in the range 2,500° C to 3,000° C will be negligible.

In this investigation specimens were ground and dispersed by ultrasonic irradiation in a water/ethanol mixture and then prepared on thin carbon fibres for examination in a Philips *EM*300 electron microscope with an anticontamination device. Electron diffraction observations and normal bright and dark field microscopy indicated improved orientation of the crystallites in the boron-treated specimen, a decrease in void content and an increase in crystallite size and perfection; detailed observations together with supporting X-ray diffraction evidence will be published elsewhere[8]. Direct resolution of the layer planes in both boron-free and boron-treated carbon fibre fragments was achieved by beam tilting[9] so that the zero order and {002} reflexions were symmetrical about the objective axis. After cutting off other reflexions with a 50 μm aperture and correcting astigmatism by reduction of the phase contrast background at very high magnification, through focus series were recorded with $\Delta f = 125$ Å at a magnification of × 200,000.

Fig. 1 is the optimum phase contrast image for a typical boron-free carbon fibre fragment. The layer planes (separation 3·4 Å) are discontinuous and imperfect, giving rise to (dark) extinction bands where they are distorted out of planes parallel to the incident electron beam.

Fig. 2 is the optimum image of part of a typical boron-treated carbon-fibre fragment. Recrystallization has occurred to give a more extensive and perfect lattice than is found in a normal carbon fibre. Extinction bands again mark lattice changes or defects; for example, the band BB delineates a region where the lattice tilts approximately 3°, and TT indicates a region where the lattice appears to twist giving rise to an area where the planes cannot be seen. These images provide additional information for the schematic model proposed earlier by Johnson and Tyson[3] for PAN based carbon fibres annealed at high temperatures. In addition, the suggestion that the subgrain boundaries between the crystallites are comprised of both tilt and twist components is substantiated by direct observation of the frequent layer plane discontinuities.

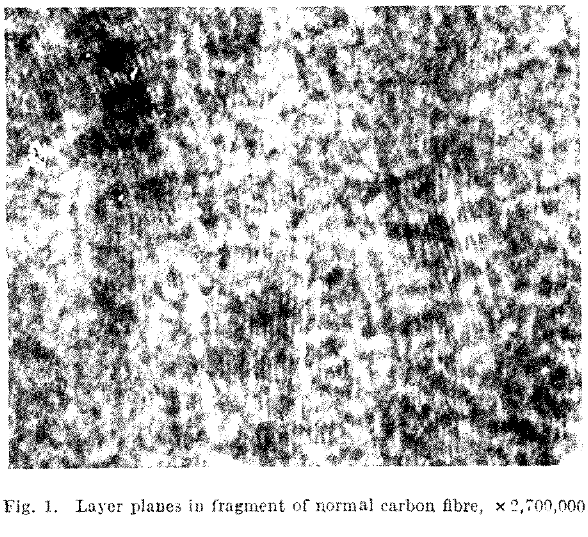

Fig. 1. Layer planes in fragment of normal carbon fibre, × 2,700,000.

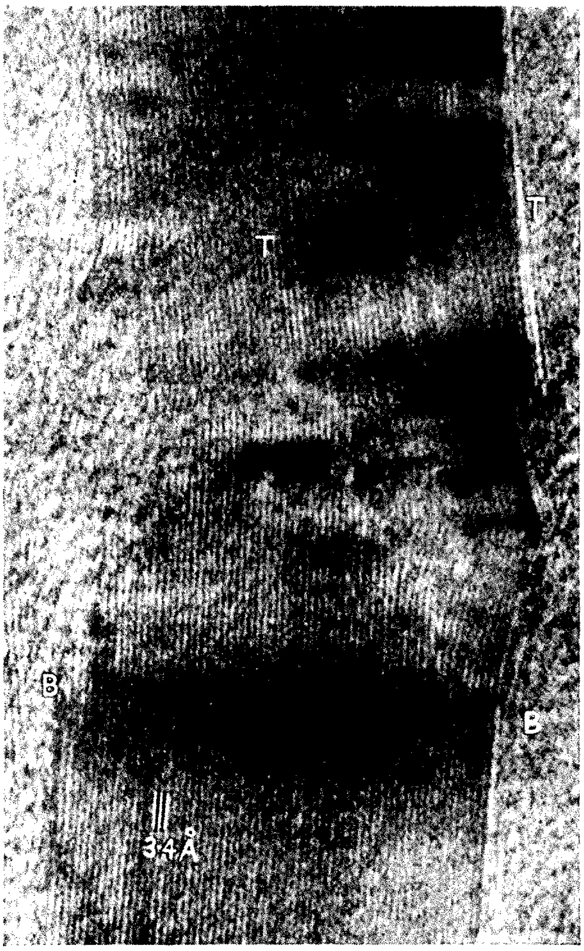

Fig. 2. Layer planes in fragment of boron-treated carbon fibre. × 2,700,000. Lattice spacing is 3·4 Å.

The increased Young's modulus and electrical conductivity reported by Allen, Cooper and Mayer[7] for boron-treated fibres can now be seen to be a result of improved orientation and perfection of the crystallites; clearly direct-lattice resolution of layer planes is a useful supplement to existing electron-microscope and X-ray diffraction methods used for microstructural investigations of carbon fibres.

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BIOLOGICAL SCIENCES

Is DNA Polymerase involved in DNA Degradation following Ionizing Radiation?

Exposure of microorganisms to ultraviolet or ionizing irradiation results in a partial degradation of their DNA. In most bacterial strains irradiated with ultraviolet light the depolymerization is not very extensive and is usually considered to be part of the DNA repair systems[1,2]. This process excises the mismatched regions of a DNA molecule containing pyrimidine dimers and replaces them by a new stretch of correctly paired nucleotides. The degradation of DNA in X or γ-irradiated microorganisms is more drastic[3,4], and parallels in extent both the radiosensitivity of bacterial strains and the radiosensitivity of their growth phases[5]. This suggests that in the case of ionizing irradiation DNA breakdown is not a part of the repair process. Moreover, the main lethal events in X-irradiated bacteria are probably internucleotide chain breaks in DNA[6,7] rather than the formation of mismatched regions. There is thus no need for such a drastic degradation before rejoining of the newly formed ends. The repair replication of DNA observed in ultraviolet-irradiated microorganisms has not been detected after X-irradiation[8].

The degradation of DNA caused by ultraviolet light and that caused by ionizing irradiation might be achieved by the same biochemical mechanisms. There has been no evidence as yet, however, concerning the enzyme(s) involved in the process of DNA breakdown. Studies with γ-irradiated *Escherichia coli* have shown that the degradation is catalysed by pre-existing enzyme(s)[5]. To learn more about this enzyme we have studied the type of enzymic attack on DNA in irradiated cells. ^{3}H-thymidine labelled material isolated at various times after γ-irradiation, denatured by alkali, and then chromatographed on a column of 'Sephadex *G*-200', gave rise to two peaks only: one corresponding to high molecular weight DNA and the other to very low molecular weight products[9]. We have been unable to detect any fragments of intermediate molecular weight by chromatography on 'Sepharose 2B' (unpublished results). These data indicate that an exonuclease is responsible for the degradation. Additional support for the exonucleolytic character of the degradation can be found in an early observation of Stuy[3], who observed that the rate of decrease in transforming activity equals that of decrease in the amount of DNA in X-irradiated *Haemophilus influenzae*.

Our analysis of the final breakdown products released into the medium has revealed, however, the presence of some products which would not be produced by the action of an exonuclease. An exponentially growing culture of *E. coli* K12 was labelled with ^{3}H-thymidine and irradiated with 20 kr of X-rays. After incubation for 90 min after irradiation, a sample of the medium was chromatographed on a column of 'Sephadex *G*-25' (Fig. 1). Two radioactive peaks were observed. The principal peak (peak II) contained 80 per cent of the recovered radioactivity and was identical to the peak obtained with non-radioactive thymine. The identity of this radioactive compound was confirmed by paper chromatography (Whatman 'No. 1', descending) in two solvent systems: (*a*) water; (*b*) 86 per cent butanol with 5 per cent of concentrated ammonia in the bottom of the tank. Thymine was also identified as the major breakdown product by Pollard and Achey[10]. It is probably a secondary product of degradation, produced from thymidylic acid (TMP) by the action of 5′-nucleotidase, an enzyme located at the surface of bacterial cell[11]. If this is true, some 80 per cent of all radioactive breakdown products must be formed by a typical exonucleolytic action. A smaller part of the radioactive breakdown products, however, was eluted before thymine (Fig. 1, peak I). This material is neither thymidine nor TMP; its chromatographic properties indicated that it could represent a mixture of small oligonucleotides. When this material was digested with phosphodiesterase (Fig. 2) its chromatographic behaviour on the column of 'Sephadex *G*-25' was identical with that of unlabelled TMP.

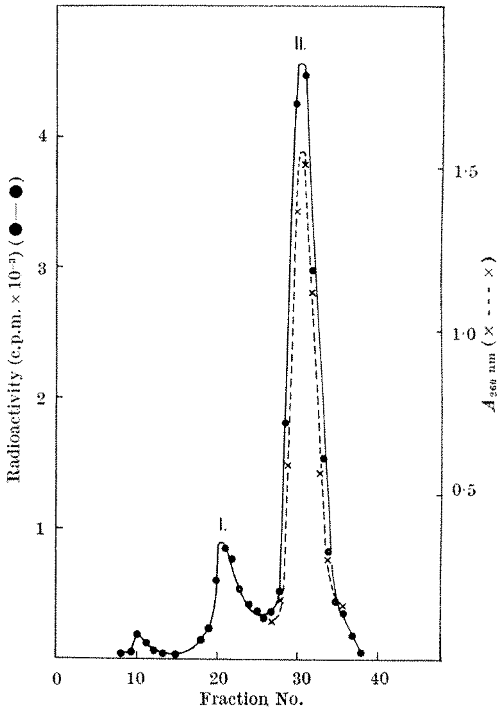

Fig. 1. Chromatography on dextran gel ('Sephadex *G*-25') of the medium containing radioactive breakdown products of DNA. 10 μCi of ^{3}H-thymidine (specific activity 3 Ci/mmole) was added to 100 ml. of a mid-logarithmic phase culture of *E. coli K*12. After 10 min the bacteria were collected, washed and resuspended in 100 ml. of fresh medium. After a further 30 min of incubation at 37° C the cells were harvested again and resuspended in 10 ml. of M/15 phosphate buffer, *p*H 7. The suspension was irradiated at 0° C with 20 kr of X-rays at a dose rate of 800 r/min. After incubation for 90 min at 37° C the bacteria were removed by centrifugation; 1 ml. of the supernatant fluid, after addition of 0·1 mg of non-radioactive thymine, was layered on the top of 31 cm × 0·87 cm² column of 'Sephadex *G*-25'. The column was eluted with 0·067 M phosphate buffer and 1 ml. fractions were collected.

The same distribution of radioactivity shown in Fig. 1 was observed when the medium was analysed 15 min after irradiation, that is, before the completion of DNA degradation in the bacterial culture. This result suggests that oligonucleotides (peak I in Fig. 1) are not intermediates in the production of the major breakdown product, thymine.

Our results demand an enzyme activity for the post-irradiation degradation of DNA which, until recently, would have been inconsistent with the mode of action of any known exonuclease of *E. coli*. Deutscher and Kornberg, however, recently described 5′→3′ exonuclease activity of DNA polymerase[12], the principal products of this hydrolysis being mononucleotides as well as oligonucleotides. Exactly the same products are formed in bacteria after ionizing irradiation, and we suggest that the hydrolytic action of DNA polymerase is responsible for their formation. The same enzyme is capable of excising the regions of DNA containing photoproducts formed by ultraviolet irradiation[13]. It has been proposed, therefore, that the polymerase plays a double part in the repair of ultraviolet-irradiated DNA: starting at a nick introduced by a specific endonuclease, this enzyme could simultaneously excise nucleotides in the 5′→3′ direction and resynthesize the degraded strand by the addition of proper nucleotides to the 3′-OH terminus formed by the nick[13]. In the case of ionizing irradiation, we must assume that the chain break, in most cases, does not produce a 3′-OH terminus; the enzyme can thus function only in the

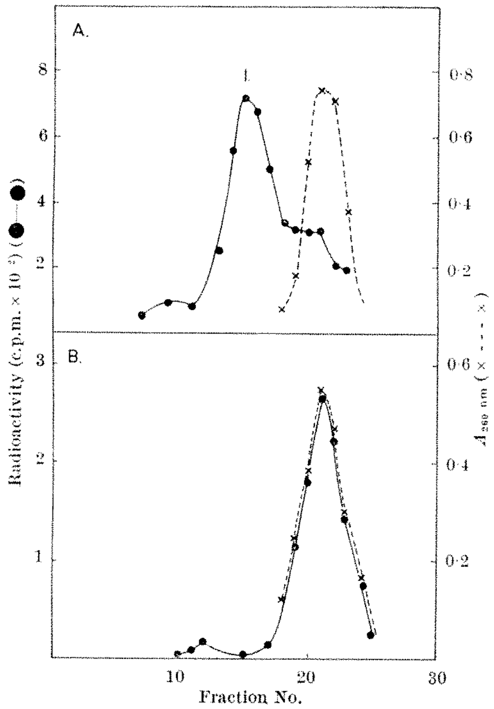

Fig. 2. Chromatography on 'Sephadex *G*-25' of the material from peak I of Fig. 1, (*A*) before and (*B*) after the digestion with phosphodiesterase. 0·1 mg of non-radioactive TMP was added to each sample. The sample (*B*) was incubated for 72 h at 37° C with an excess of phosphodiesterase from the venom of *Vipera lebetina* (a gift from Dr E. Palaček, Brno) in 0·1 M Tris-HCl (*p*H 8·5) and 0·03 M magnesium acetate. The column was eluted with 0·01 M Tris-HCl buffer (*p*H 8·5).

$5' \rightarrow 3'$ direction, as an exonuclease and not as a polymerase. Such an action would result in a final loss of viability of the irradiated cell—a phenomenon which indeed accompanies the degradation of DNA in X or γ-irradiated bacteria[5].

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Conservative Mutations in Homologous Proteins

IT has been argued that most of the evolutionary changes observed in homologous proteins are the result of chance fixation of selectively neutral mutations[1,2]. This hypothesis would be clarified by a more detailed analysis of observed patterns of amino-acid substitutions in related proteins than has been attempted previously[3].

When phenylalanine, tyrosine, proline, tryptophan, methionine, leucine, isoleucine and valine are considered hydrophobic and mutations to synonymous codons are ignored, the result of undirected or random interchanges is shown in Table 1. Epstein's conclusion[3] that the code is capable of favouring mutations between amino-acids of similar physical properties is confirmed.

Table 1. PERCENTAGES OF MUTATION RESULTING IN INTERCHANGE OF SIMILAR AMINO-ACIDS

	Number of mutations observed	Percentage similar	Probability percentage similar is different from	
			Random	Code-directed
Random*		50·9		
Code-directed*				
One step		66·3	$P < 0{\cdot}001$	
Two step		59·0	$P < 0{\cdot}05$	
Closely related proteins				
One step	298	85·7	$P < 0{\cdot}001$	$P < 0{\cdot}001$
Multiple step	116	67·2	$P < 0{\cdot}001$	$P = 0{\cdot}017$
Distantly related proteins				
One step	359	81·4	$P < 0{\cdot}001$	$P = 0{\cdot}001$
Multiple step	252	56·3	$P = 0{\cdot}089$	$P = 0{\cdot}434$

* Normalized to average amino-acid composition of proteins examined for mutations.

To examine the actual requirements of proteins for a particular amino-acid composition, several groups of closely related proteins were selected from the sequence data of Dayhoff and Eck[4]. These groups consisted of all the sequenced variants of the haemoglobin α and β chains, insulin A and B, cytochrome *c* and ferredoxin. Each mutation was classified as explicable by single or multiple base changes and as being between similar or dissimilar amino-acids[5]. Very distantly related proteins were similarly considered and the groups here consisted of (1) human haemoglobin α chain: whale myoglobin: lamprey globin; (2) lysozyme: lactalbumin α; (3) trypsinogen: chymotrypsinogen; (4) *B. subtilis* Carlsberg subtilisin: subtilisin BPN′; and (5) $\lambda_{\mathrm{Sh}}:\varkappa_{\mathrm{Cum}}$ Bence-Jones proteins. Clearly, single step but not multiple step mutations have been "conservative" in the sense that they have resulted in the interchange of amino-acids with similar physical properties.

In the single step mutations examined, the incidence of transversions was approximately twice the incidence of transitions (403:221) and this is the ratio expected if mutation is independent of codon base composition. For this reason and because frequency of point mutation in several globular proteins is predictable by a Poisson distribution[1], non-random mutations cannot be invoked to explain the high observed frequency of conservative mutations or the disproportionately large number of multiple step mutations in both closely and distantly related molecules.

If it is assumed that the sequence data used in this study are an unbiased sample of all observable variants of each of the proteins in question, then the number of interchanges at any particular kind of site become a function of the mutability of the site, the likelihood that any mutation will not be lethal and the proportion of that kind of site in the molecule. As shown above, the mutability of a site is independent of codon base composition. A reasonable estimate of the proportion of non-lethal mutations, based on studies of the histidine-C locus in *Salmonella*, is 0·90 (ref. 6).

When the frequency of mutated codons is relatively low, as in closely related proteins, the frequency of multiple step mutations should approximate the square of the frequency of single step mutations after correction for lethals. In our data the frequency of single step mutations is 0·051 and that of multiple step mutations is 0·020. The expected frequency of the latter is 0·0029 or about one-seventh the observed frequency.

A simple explanation for the high frequency of multiple step mutations can be made by initially assuming that all mutations occur at "restricted" sites, requiring only a polar or a hydrophobic residue. In this case 15 per cent of the sites would be unrestricted in closely related proteins, to be consistent with the experimental data. A lower limit can be established by assuming that one-fifth of single step and all multiple step mutations occur at sites requiring no particular type of residue, that is, at unrestricted sites. In these conditions 0·6 per cent of sites are unrestricted. These limits are consistent with those of King and Jukes[1] but, because we feel that the latter figure is more nearly correct, our estimate tends to be lower.

Here the concept of selectively neutral mutation is fundamental, because the interchanges occurring at unrestricted sites seem to be almost equivalent selectively. It has been demonstrated many times since Fisher[7] that an arbitrarily small selective advantage or disadvantage may be undetectable in a single individual but quite apparent in a sufficiently large population. In this context, any mutation will be considered other than neutral when a sufficiently sophisticated examination of its effects is made. It is not yet possible to assess the neutrality of such mutations at a population level[6]. It is certainly not valid to maintain that a mutated protein is equivalent to the wild type on the basis of such gross evaluation as enzyme activity assays or the absence of clinical symptoms.

Furthermore, with the exception of the haemoglobin variants, all of the proteins used in this study are the most common alleles present in a particular species, so that one cannot assume that any other sequences would be functionally equivalent. That homologous sequences are functional is not strictly relevant because the two molecules are very likely to function in quite different internal environments and many compensatory mutations may have occurred even within the same molecule.

The data presented suggest that most single step mutations fixed during specification are conservative in that interchanges are observed between amino-acids of similar physical properties. While about 65 per cent of all positions must be occupied by either a hydrophobic or polar residue, between 0·6 per cent and 15 per cent of the mutable sites may not require a polar or hydrophobic amino-acid in a protein which is functional in some circumstances. Mutations occurring at these sites are not shown to be selectively neutral, and although their selective effect may be small it is not likely to be insignificant at a population level. These data should therefore not be used to support the concept of selectively neutral mutation.

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Pre-cell Evolution and the Origin of Enzymes

SALISBURY[1] has asked how enzymes could arise by natural selection from less developed ancestors if the minimal structural requirements for their activity are so inflexible that variants below a certain high level of development cannot exist. This problem can be resolved by considering the evolution of catalytic rates.

Koshland and Neet[2] have estimated that typical enzymes of present-day cells catalyse reactions at rates 10^{14} to 10^{18} times those which might be expected without a catalyst. I assume that these great activities were achieved in gradual steps, largely during the period of chemical evolution before cells existed. It would then seem reasonable that an enzyme which multiplies the rate of a particular reaction by a factor of 10^{14} may have originated as a polypeptide with a corresponding catalytic factor for the same reaction of about 10^3. If a mole of this enzyme now produces one mole of product in 10^{-2} s, it would originally have produced one mole in about thirty years. An inescapable implication of this calculation is that the earliest enzymes had catalytic activities far below the lowest levels of detection by known techniques. It is further implied that the first self-replicating pre-cell structures reproduced with extreme slowness, perhaps only once in hundreds or even thousands of years.

I now assume that speed and specificity are intimately linked in their evolution, for high rates are undoubtedly attained by close fitting of polypeptides to particular substrates. I thus propose that the first generation of enzymes consisted of random polypeptides, each of which could catalyse many reactions at extremely low rates, and that they gave rise through natural selection to individual types which were progressively more specific and faster acting. These considerations suggest a very simple first self-replicating unit, which may have formed and evolved according to the hypothetical series of events outlined in the model described below.

In the beginning millions of molecules of pre-existing random polynucleotide-like substances are slowly replicated[3,4] with the non-specific catalytic aid of pre-existing polypeptide-like polymers[5]. Through a stereochemical coding mechanism[6–10], so far undemonstrated, and polypeptide catalysis, new peptides are also formed on the polynucleotide templates. Among the original templates, I assume that one codes for a polypeptide with "nucleic acid polymerase" activity which, though still extremely small by present standards, is about ten times greater than that of the product of any other template of the period. Because of the proximity of this polynucleotide and its daughter polynucleotides to molecules of the new polymerase, further copies of the template will be made at an accelerated rate and it will therefore increase in number relative to all others. I now suppose that, associated with one of the numerous members of later generations of this polynucleotide, a new sequence of nucleotide residues occurs which codes for a polypeptide with "protein polymerase" activity greater than usual for that time, though also still very small. Clearly the two polymerase templates, functionally united with their polypeptide products, would constitute a self-replicating unit that could increase in number logarithmically in an adequate medium and far outstrip its competitors. Through step-by-step repetition of mutation and selection the two enzymes would become more rapid in action and more specific. Other enzymes, as well as structural proteins which facilitate reproduction, would be developed similarly.

An important aspect of this scheme is that the stability of the complexes formed during the first template-directed syntheses of proteins and nucleic acids may have obviated the requirement for activation of the substrates when the process was very slow. Another point is that the mutation rate should have been very great at first, diminishing as the enzymes were perfected and the accuracy of transcription was correspondingly increased.

The possibility that processes now completed in milliseconds once required hundreds of years suggests that the dominant driving force of pre-cell evolution was the demand for ever-increasing rates of multiplication to ensure survival, in contrast to the demand for adaptation which characterizes the Darwinian period. Awareness of the extreme slowness of the first developmental steps could simplify conceptual problems, but it casts a somewhat discouraging shadow on prospects for understanding these early events through laboratory experiments of the kind now in progress. A similar and related slowness seems to frustrate efforts to elucidate the separate functions of the individual parts of fully developed enzymes[11].

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Enhanced Binding of FAD to Glutathione Reductase in G6PD Deficiency

THE activity of glutathione reductase (GR) in erythrocytes deficient in glucose-6-phosphate dehydrogenase (G6PD) is approximately 1·5 to 2 times higher than in red cells with normal G6PD activity[1]. This may provide a compensatory mechanism for the defect in glutathione reduction in G6PD deficient cells. The cause of the increased activity of GR in G6PD deficiency is unknown and the possibility of increased production of GR in erythrocyte precursors of G6PD deficient individuals seems unlikely. The lower mean red cell age, resulting from a moderately shortened life span of G6PD deficient erythrocytes[2], may play a part but is unlikely to account entirely for the marked increase in GR activity. Recently Beutler[3] reported a stimulation of erythrocyte GR in normal subjects by flavin-adenine dinucleotide (FAD) *in vitro* and by supplementation of its precursor riboflavin *in vivo*. GR activity was shown to depend on dietary intake of riboflavin, but even in persons with a relatively high intake of this vitamin GR does not seem to be saturated with FAD[4]. These findings suggest that differences of FAD binding of GR may provide an explanation for the increased GR activity in G6PD deficient red cells.

We have recently examined GR activity in haemolysates of three groups of subjects with normal and deficient G6PD without and with addition of FAD to the test mixture (final concentration 1·2 μM) and before and after riboflavin supplementation (10 mg per day for 8 days). The GR assay mixture contained 50 mM Tris buffer (*p*H 7·5), 0·3 mM NADPH, and 3 mM GSSG; the reaction temperature was 25° C; units correspond to a substrate turnover of 1 μmole/l. per min and 10^{11} erythrocytes. The three groups of subjects are (1) seven Europeans living in northern Thailand, (2) twelve healthy adult Thai males with normal erythrocyte G6PD activity, and (3) ten Thai males with G6PD deficiency who live in the same environmental (in particular nutritional) conditions as group (2). All individuals in group (3) had G6PD activities of less than 10 per cent of the average level of Thai males with normal G6PD activity; they were not anaemic and numbers of reticulocytes were only slightly increased (1·8 to 3·2 per cent). In the Europeans GR activity was 10·6 ± 1·8 units, a value similar to that observed in a large group from Germany[5]. The results in the Thai groups are shown in Table 1.

Table 1. GR IN ERYTHROCYTES OF SUBJECTS WITH NORMAL AND DEFICIENT G6PD. EFFECT OF ADDITION OF FAD AND SUPPLEMENTATION OF RIBOFLAVIN

Group	GR activity before riboflavin supplementation		
	Without FAD	FAD added	Increase with FAD (per cent)
G6PD normal	8·9 ± 2·1	14·8 ± 1·8	66·8 (25·8–133·3)
G6PD deficient	15·8 ± 2·2	16·6 ± 1·9	5·7 (0–12·1)
	GR activity after riboflavin supplementation		
	Without FAD	FAD added	Increase with FAD (per cent)
G6PD normal	15·1 ± 2·1	15·6 ± 1·8	3·6 (0–10·5)
G6PD deficient	16·6 ± 1·8	17·0 ± 1·7	2·3 (0–6·7)

In the group with normal G6PD activity the mean level of GR activity is lower than that in Europeans, possibly reflecting a lower dietary supply of riboflavin. Addition of FAD to the test mixture results in a considerable increase in GR activity (average 67 per cent). After administration of riboflavin, GR activity without added FAD is similar to that originally observed with added FAD, and the rise caused by addition of FAD is now minimal. In the group with G6PD deficiency original GR activity is, on the average, 78 per cent higher than in the group with normal G6PD. Addition of FAD and riboflavin supplementation result in only a small increase in GR activity.

The results indicate that FAD binding of GR is much closer to saturation in erythrocytes of subjects with G6PD deficiency living in the same environmental conditions as a control group with normal G6PD. Although maximal GR activity after supplementation of riboflavin and addition of FAD is lower in the group with normal G6PD (15·6 compared with 17·0 units), the probability for a chance difference is relatively high ($0\cdot1 > P > 0\cdot05$). This suggests that increased binding of FAD by GR accounts for most of the elevation of GR activity in G6PD deficient erythrocytes in normal nutritional conditions. If a significant difference of maximally stimulated GR activity between subjects with normal and deficient G6PD emerges from a larger sample, this relatively small additional increase would be most likely attributable to the younger red cell population in G6PD deficiency. Increased absorption of riboflavin by G6PD deficient individuals or a stuctural difference of GR in erythrocytes with normal and deficient G6PD seem improbable. Thus it seems likely that the metabolic anomalies of G6PD deficient red blood cells cause an elevation of FAD concentration or facilitate the binding of FAD by the GR enzyme.

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Hybrid Molecules of Yeast and Rabbit GPD containing Native and Modified Subunits

CONVINCING evidence has been presented that molecules of glyceraldehyde-3-phosphate dehydrogenase (GPD) from yeast[1] and from rabbit skeletal muscle[2] each comprise four identical polypeptide chains and bind a maximum of four molecules of NAD per molecule of enzyme[3–5]. But whereas the binding of NAD to the yeast enzyme (Y_4) at 40° C is considered[3,4] to conform to the Monod, Wyman and Changeux model[6] for allosteric interactions, which requires highly cooperative structural transitions of the subunits within a given enzyme molecule, the binding of NAD to rabbit GPD (R_4) shows a negative cooperativity in that binding of successive molecules of NAD to a given GPD molecule diminishes the affinity for NAD of the remaining unoccupied sites[5]. As such the rabbit enzyme conforms to the "stepwise" model for ligand binding proposed by Koshland, Nemethy and Filmer[7].

Because the differences in behaviour of NAD binding presumably reflect differences in interchain interactions in the yeast and rabbit enzymes we were interested to attempt the preparation of hybrid molecules of GPD comprising subunits from both enzymes. We describe here the preparation of such hybrids as well as of hybrids in which two of the four subunits are covalently substituted at the active centre with 2-bromoacetamido-4-nitrophenol[8] (Koshland's "reporter" group).

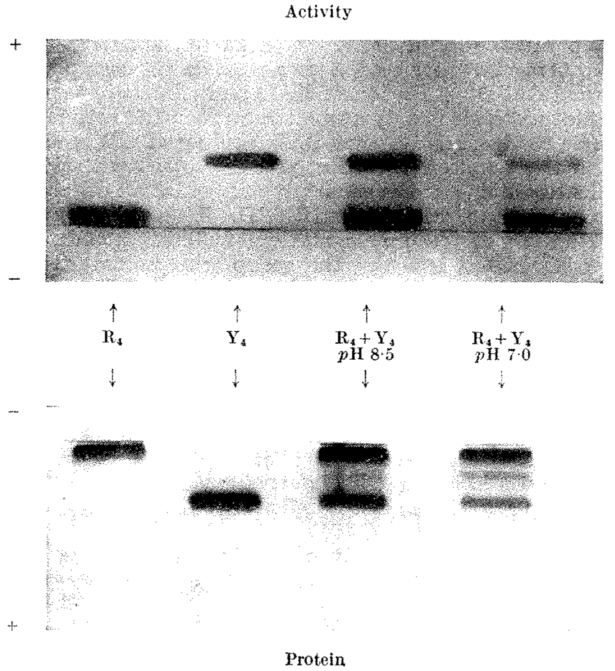

Fig. 1. Starch-gel electrophoresis of a hybridized mixture of yeast and rabbit GPD. Solutions containing both yeast (Y_4) and rabbit (R_4) GPD at a concentration of 3 mg/ml. were dialysed at *p*H 7·0 or 8·7 in the conditions described in the text. Electrophoresis was carried out for 4 h at 30 V/cm using the system of Rosemeyer and Huehns[15]. The upper photograph shows one half of the gel stained for enzyme activity and the lower photograph the other half stained for protein. Enzymically active bands in the position expected for the R_2Y_2 hybrid are obtained after hybridization at both *p*H values.

Fig. 1 depicts the results of starch gel electrophoresis of mixtures of yeast and rabbit GPD (each containing approximately four molecules of bound NAD per enzyme molecule), previously dialysed for 14 h at 4° C against either 0·01 M citrate–0·001 EDTA at *p*H 7 or 0·01 M Tris-0·005 M borate–2×10^{-4} M EDTA at *p*H 8·7, the latter buffer being that used in the electrophoresis. In each case an additional enzymically active band is observable with a relative migration of 0·54 compared with a value of 1·00 for the yeast enzyme (Y_4) and 0·16 for the rabbit enzyme (R_4). The relative migration of R_3Y, R_2Y_2 and RY_3 hybrids would be 0·79, 0·58 and 0·37, respectively, given that the size and shape of the yeast and rabbit enzymes are identical and differ only in charge, so that the single new band with a relative migration of 0·54 is identifiable as the R_2Y_2 hybrid. Note that bands in the positions expected for RY_3 or R_3Y hybrids have not been observed in the course of more than sixteen hybridization experiments covering a range of protein concentrations from 1·0 to 4·0 mg/ml.

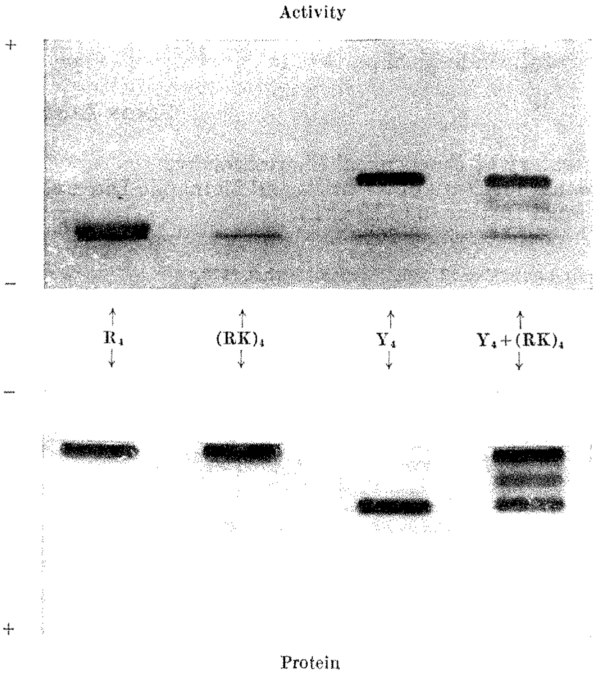

Fig. 2. Starch-gel electrophoresis of a hybridized mixture of yeast and modified rabbit GPD. The conditions were as for Fig. 1 except that the yeast GPD (Y_4) was hybridized with rabbit GPD modified by reaction with Koshland's reagent, $(RK)_4$. The $(RK)_4$ derivative is shown to be enzymically inactive but the $(RK)_2Y_2$ band is clearly active.

The ability to make R_2Y_2 hybrids allows in principle the preparation of hybrids in which two subunits have been covalently substituted. Thus yeast GPD (Y_4) was hybridized with rabbit GPD which had been previously inactivated by substitution with Koshland's reagent[8] at four sites per tetramer [$(RK)_4$] (ref. 9). This derivative has been shown to be modified at the four "rapidly reacting" thiol groups of GPD (refs. 9 and 10). After hybridization a single new species (Fig. 2) was again observed with a relative migration value of 0·59 ($Y_4=$ 1·00). Furthermore, this hybrid was enzymically active with G3P as substrate, indicating that yeast chains are catalytically active even when combined with inactive rabbit chains. As in the case of the Y_4+R_4 hybridizations, only one type of hybrid was observed, migrating in a position near that expected for a $(RK)_2Y_2$ hybrid.

In a separate experiment, the $(RK)_2Y_2$ hybrid was rapidly eluted from the starch gel and assayed with glyceraldehyde 3-phosphate as substrate before reversion to $(RK)_4$ and Y_4 occurred. It was found that the $(RK)_2Y_2$ hybrid had a specific activity of 45 per cent of the native yeast enzyme, indicating that combination with the inactive rabbit chains has little effect on the catalytic activity of the yeast subunits.

The failure to observe RY_3 and R_3Y hybrids in the conditions of this study indicates that the molecules of yeast and rabbit GPD must be regarded as isologous[6] rather than heterologous[6] tetramers (see Fig. 3). This conclusion is consistent with: (*a*) the finding that the molecules of lobster[11] and human[12] GPD have two-fold

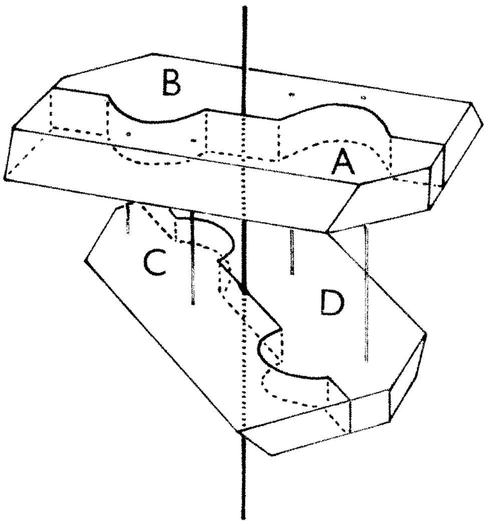

Fig. 3. Diagrammatic representation of an "isologous" tetramer comprising four identical subunits. The structure has three mutually perpendicular two-fold axes of rotation, one being shown as the dark vertical line. The A–B, A–C and A–D interactions are non-equivalent.

symmetry axes, (*b*) the fact that the rabbit muscle enzyme dissociates to dimers on dilution[13], and (*c*) the observation that only two equivalents of furylacryloyl groups could be introduced per molecule of rabbit or yeast GPD (ref. 14).

The formation of hybrid molecules from enzymes taken from such widely differing organisms as yeast and the rabbit indicates that the nature of the (AB)–(CD) interaction must be similar in each enzyme (see Fig. 3). But it seems that this interaction, although similar, is probably not identical, for in that case it would be anticipated that an equilibrium mixture formed from equal amounts of rabbit and yeast GPD would contain R_4, R_2Y_2 and Y_4 in the ratio 1 : 2 : 1. Inspection of Figs. 1 and 2 shows that such a ratio is not attained, indicating either that equilibrium had not been reached in these experiments or that the inter-dimer interaction of R_2 with Y_2 in R_2Y_2 is weaker than that of one or both of the (R_2)–(R_2) or (Y_2)–(Y_2) interactions in R_4 or Y_4. We now seek to determine the kinetic and thermodynamic aspects of hybrid formation as well as the chemical properties of the native and modified hybrid.

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Lymphocyte Activation: a Rapid Test to predict Allograft Rejection

THE early detection of allograft rejection, before organ function deteriorates, is an important objective of clinical research in transplantation immunology. Because lymphocytes play a central part in graft rejection[1,2], attention was focused on these cells in the circulation of graft recipients. We observed that the numbers of circulating lymphocytes that incorporate ^{3}H-thymidine rise significantly during organ allograft rejection and may predict rejection crises by 24–48 h.

The ^{3}H-thymidine incorporation of the circulating lymphocytes of organ allograft recipients was studied serially. Peripheral blood was collected by venipuncture, defibrinated by swirling with glass beads and the red blood cells sedimented with 0·4 per cent dextran. The resultant leucocyte rich serum was adjusted with the patient's own cell-free serum to a final concentration of 10^6 lymphocytes per ml. 1 ml. of this cell suspension and 2 ml. tissue culture media were incubated in quadruplicate in 13×100 ml. round bottom tubes in a water bath at 37° C for 30 min. 2 μCi of ^{3}H-thymidine was then added and the tubes incubated for a further 2 h. The cells were then washed, treated with 10 per cent trichloroacetic acid and the acid-precipitable radioactivity counted in a liquid scintillation counter. Results were recorded as the average counts per minute per 10^6 lymphocytes (c.p.m.) of the four samples.

Table 1. CORRELATION BETWEEN ACTIVATED LYMPHOCYTES AND CLINICAL DATA

Days after graft	24 h urine output (ml.)	Serum creatinine (mg per cent)	Azathioprine (mg/d)	Methyl prednisolone (mg/d)	ALG (mg/d)	Activated lymphocytes c.p.m./10^6 lymphs
0	1,038	9·2	350	125	0	102
1	1,842	7·2	200	120	0	N.D.
2	2,829	2·3	250	80	0	N.D.
3	1,230	N.D.	200	80	0	223
4	800	1·6	100	80	0	1,102
5	104	1·9	50	60	0	757
6	30	5·0	200	200	75	2,107
7	120	7·5	200	240	150	1,027
8	65	12·4	200	200	300	2,038
9	633	11·7	200	240	300	N.D.
10	1,184	13·8	200	240	350	2,422
11	1,490	7·8	200	240	350	780
12	2,405	N.D.	200	160	350	980

Fifteen normal subjects served as controls. The ^{3}H-thymidine of their circulating lymphocytes was low (median: 270 c.p.m.; range: 92–1,420 c.p.m.).

Nine potential allograft recipients were studied before transplantation. The thymidine incorporation of their circulating lymphocytes was also low (median: 225 c.p.m.; range: 47–859 c.p.m.).

The nine patients subsequently received an organ allograft, seven renal and two cardiac. Serial studies after transplantation revealed that, in six of the subjects, the ^{3}H-thymidine incorporation of their circulating lymphocytes rose to over 2,000 c.p.m. per 10^6 lymphocytes. The median of the six was 4,100 c.p.m. with a range of 2,100–15,000 c.p.m. Each rise occurred during the three week period after the transplant, was correlated with an episode of graft rejection and usually preceded clinical evidence of graft rejection by 24–48 h. A representative case is shown in Table 1. When the data were corrected so that the results were expressed as c.p.m. per total circulating lymphocyte population, the rise was still seen. Even though the patients usually became very lymphopenic after transplantation, there was thus a 6·2 to 7·8-fold increase in the absolute number of circulating thymidine incorporating cells. In three patients an attempt to reverse rejection by increasing the dosage of immunosuppressive therapy was associated with a prompt fall in the ^{3}H-thymidine incorporation of the patients' circulating lymphocytes.

The appearance of ^{3}H-thymidine incorporating large lymphoblasts has been observed in nodes draining the graft bed before graft rejection in animals[1]. The appearance of these cells heralds the development of immunological commitment to the graft antigens. During effective immunosuppression the development of committed effector lymphocytes from these cells is inhibited[2]. During the immune response to antigenic stimuli, including grafts, large numbers of these large lymphoid cells are produced by proliferation, are released into the efferent lymph, enter the blood and ultimately invade the graft bed where they act as effectors of cellular immunity[3]. This phenomenon also occurs during the response of man to typhoid vaccine and the cells can be detected in the peripheral blood by morphological examination or by measurement of thymidine incorporation[4].

On the basis of these findings, we postulated that the activated lymphocytes would appear in the circulation and could be detected during or even preceding clinical evidence of graft rejection. The experimental data support this hypothesis and suggest that the serial measurement of peripheral blood activated lymphocytes will provide data which can predict by 24–48 h an episode of organ allograft rejection.

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Blood Group H Antigen of Influenza A2 Virus

McDonald and Zuckerman[1] have demonstrated statistically that there are a large number of blood group O patients and a relatively few of group A among those infected with influenza A2 virus. Subsequent studies[2–4] have indicated, however, that an antibody against influenza was more commonly found in sera of group O persons than group A. Potter and Schild[2] suggested that such a divergency in influenza infection was influenced by genetic factors related to ABO blood groups. We have examined influenza viruses for blood group antigens in order to obtain evidence for any possible relationship between influenza and ABO blood groups.

We used allantoic fluid from hens' eggs inoculated with influenza A, A2 or B virus. The fluid was centrifuged at low speed to remove membrane debris and centrifuged for a further 3 h at 100,000*g* to sediment viruses; the sediment was washed by resuspending it in buffered saline (*p*H 7·0) and then centrifuging. The final sediment was suspended homogeneously in buffered saline, and its agglutinating titre with chicken red cells adjusted to 1 : 2,048 by dilution. The suspension was heated for 20 min in boiling water. Preliminary tests of agglutinin inhibition showed no decrease in titre of anti-A, anti-B and anti-H agglutinins. The suspension was then examined by an absorption–elution test in the following way. Thin pieces of paper (3 × 5 mm) were dipped into a serially diluted row of the suspension, containing 2·5 per cent hen's egg albumin, and dried at room temperature. The papers were immersed in acidified methanol for 20 min and dried for 4 h at 56° C; they were then placed in 1 ml. of potent anti-H reagent (extract of *Ulex europaeus* seed) and allowed to stand overnight at 2° C, after which time they were washed four times in a sufficient volume of normal saline. After the addition of normal saline (0·09 ml.) the papers were heated at 50° C for 20 min in a water bath. Eluted agglutinin in the normal saline was separated and a serial dilution was made immediately.

Table 1. COMPARISON OF RESULTS OF ANTI-H ABSORPTION–ELUTION TESTS OF INFLUENZA A, A2 AND B VIRUSES

Virus	Titres of anti-H obtained by tests of undiluted samples	Minimum virus* concentrations to detect anti-H
Influenza A	< 1	> 2,048
Influenza A2 (Kumamoto)	8	64
Influenza A2 (Hongkong)	8	128
Influenza B	1	2,048
Control	< 1	—

* Represented by titres of chicken red cell agglutination.

Agglutinin tests of the eluate were performed by the immediate addition of a 1/2 part of 0·5 per cent red cell suspension (group O) to the diluted row and by low speed centrifugation after addition of 1/2 part of agglutinin-free human group AB serum following 15 min storage at 5° C. Tests of papers adsorbing the undiluted suspension of influenza A2 viruses gave relatively high titres of anti-H activity (Table 1); the influenza A virus proved to have a trace of anti-H in an eluate; the B virus gave a weakly positive reaction. The minimum concentrations of the viruses required for the detection of anti-H were low for A2 viruses and high for A and B. A control experiment was simultaneously performed using paper adsorbing non-inoculated egg allantoic fluid plus 2·5 per cent egg albumin, but the results of absorption–elution were negative. Detection of blood group A and B antigens was also performed, but the results obtained were not reliable, because in some experiments anti-A and anti-B were eluted from papers with fixed egg albumin only.

Our results establish the presence of blood group H antigen in influenza A2 and B viruses, the quantity being more in A2 than in B. We conclude that anti-H agglutinin is involved in the prevention of certain influenza infections. The relatively high incidence of group O patients infected with some types of influenza may be attributable to the lack of anti-H antibody in their sera or secretions.

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Relationship between Glutathione Reductase Activity and Drug-induced Haemolytic Anaemia

THE maintenance of glutathione in the reduced state in red cells is achieved through the following three metabolic steps

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + \text{ADP} \qquad (1)$$

$$\text{Glucose 6-phosphate} + \text{NADP} \xrightarrow[\text{dehydrogenase}]{\text{glucose 6-phosphate}} \text{glucono-}\delta\text{-lactone 6-phosphate} + \text{NADPH} \qquad (2)$$

$$\text{GSSG} + \text{NADPH} \xrightarrow[\text{reductase}]{\text{glutathione}} 2\ \text{GSH} + \text{NADP} \qquad (3)$$

In glucose 6-phosphate dehydrogenase deficiency, metabolism in the second step of the sequence is impaired. As a consequence, when treated with acetylphenylhydrazine *in vitro*, the red cells form increased numbers of Heinz bodies[1] and show instability of GSH. *In vivo*, they are susceptible to haemolysis by drugs such as primaquine, nitrofurantoin, phenylhydrazine and sulphonamides[3].

The consequences of a defect in the third step of this sequence, catalysed by glutathione reductase, are not yet clear. It has been held that glutathione reductase deficiency, like glucose 6-phosphate dehydrogenase deficiency, results in increased susceptibility to the haemolytic effects of oxidative drugs[4-7]. But because the level of glutathione reductase in such patients is only decreased to about one-half of the normal value, the aetiological role of glutathione reductase deficiency in the haemolytic episodes reported has been questioned[8].

It has been found recently that red cell glutathione reductase activity depends largely on dietary riboflavin intake[9-11]. Deficiency of this enzyme can be corrected within a few days by the oral administration of only 5 mg riboflavin[11] or 10 mg flavin mononucleotide daily[12]. This has made it possible for us to examine the role of glutathione reductase deficiency in the origin of drug-induced haemolytic anaemia.

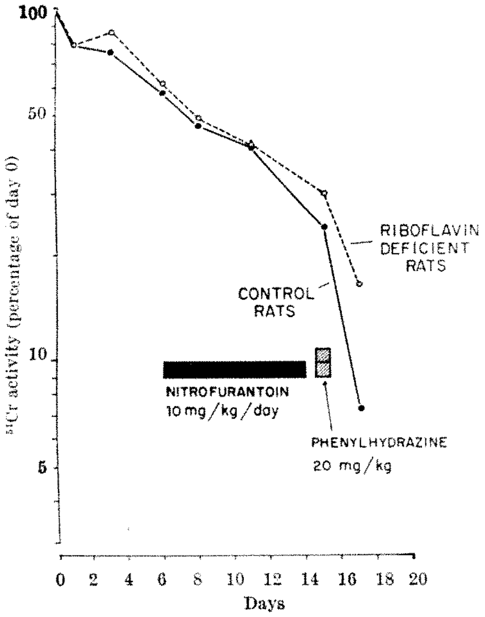

Fig. 1. The effect of nitrofurantoin and phenylhydrazine on the survival of ^{51}Cr labelled erythrocytes in glutathione reductase deficient (riboflavin deficient) and control rats. The points represent average values obtained from four riboflavin deficient and two control rats.

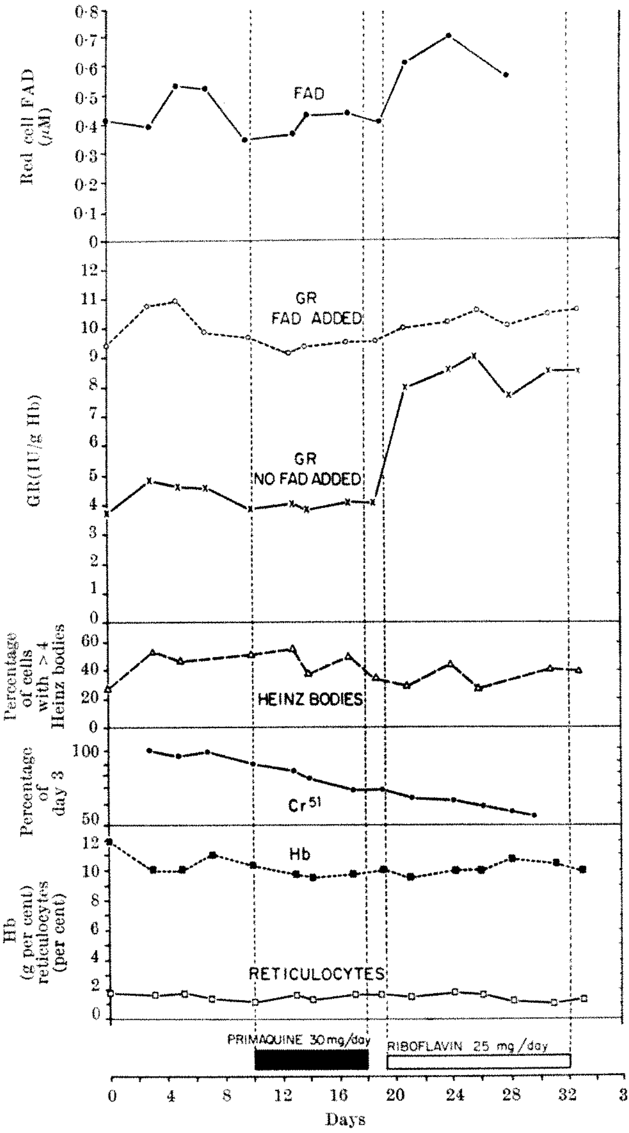

Fig. 2. The effect of primaquine and riboflavin on the erythrocyte of glutathione reductase (GR) deficient (riboflavin deficient) subject.

Preliminary studies were carried out on four rats on a riboflavin-free diet (Nutritional Biochemicals). After 5 weeks, glutathione reductase levels in the red cells of the riboflavin deficient rats were found to average 1·8 international units/g haemoglobin at 37° C, which was 31 per cent of the level in the red cells of rats on the same diet supplemented with 20 mg of riboflavin/kg of diet. Glutathione reductase assays were carried out as previously described[10]. ^{51}Cr survival curves were carried out before and during the administration of 10 mg nitrofurantoin per kg of body weight daily for 8 days. No change in the ^{51}Cr red cell survival curve was found (Fig. 1). Each rat was then given a single dose of 20 mg phenylhydrazine-HCl per kg body weight subcutaneously. The fall in ^{51}Cr activity of the blood indicated that this dose produced rapid red cell destruction, but the rate of red cell destruction was no greater in riboflavin deficient than in normal animals.

We then studied a Negro female (N.L.-2) with sickle cell trait and very mild iron deficiency anaemia. She has been known to have red cell glutathione reductase levels diminished to approximately 60 per cent of normal for several years[11]. She was given a diet containing between 0·5 and 0·6 mg of riboflavin daily, and a ^{51}Cr red cell survival study was initiated. There was no change in the slope of the ^{51}Cr survival curve when 30 mg of primaquine base was given daily for 8 days. The patient was then given 25 mg of riboflavin daily, and a rise in her red cell glutathione reductase activity and flavin adenine dinucleotide (FAD) levels was noted. There was no change, however, in ^{51}Cr survival, haemoglobin level, reticulocyte count or Heinz body test[1] (Fig. 2).

Our findings show that if levels of glutathione reductase are decreased to 40–60 per cent of normal, sensitivity to the haemolytic anaemia induced by oxidant compounds does not ensue. Either the glutathione reductase activity as measured in the haemolysate does not represent the *in vivo* capacity of the red cell to reduce GSSG, or, more likely, the glutathione reductase activity does not represent the limiting step in the sequence of reactions 1–3 which are required. We assume that in those patients reported to have drug-induced haemolytic anaemia with glutathione reductase activities 50 per cent of normal, factors other than the glutathione reductase level must have been important in the aetiology of the haemolytic anaemia.

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Lanthanum Inhibition of ^{45}Ca Efflux from the Squid Giant Axon

LETTVIN *et al.*[1] predicted that lanthanum ions, because of their relatively small size and high charge density, should have a much greater electrostatic attraction for any negative calcium binding site than calcium ions themselves. This was borne out for the membranes of the barnacle muscle fibres[2] and the lobster axon[3]. If negatively charged sites located in the membrane also serve to transport calcium ions across it, then lanthanum ions would be expected to displace calcium from these sites, and thereby inhibit the calcium ion flux. It has been shown that this is true for an artificial membrane with negative groups of phospholipids lining aqueous pores[4]. Data obtained by Weiss and Goodman[5] and Van Breemen[6] indicate that lanthanum ions also block the calcium transport across smooth muscle membranes. In smooth muscle physiology there is much uncertainty about the relative contribution of calcium influx to the initiation of contraction, and a calcium transport blocking agent would be very useful. In addition, information regarding the nature of the lanthanum blockade should contribute to the understanding of the cell membrane calcium transport system itself.

Baker *et al.*[7] demonstrated that lanthanum inhibited the lithium-sensitive sodium efflux which is coupled to calcium influx. Blaustein and Hodgkin[8] have shown that part of the calcium efflux from the squid axon is coupled to calcium influx and part to sodium influx. Evidence for calcium exchange diffusion has also been presented for smooth[9], cardiac[10] and skeletal[11] muscle, and a phospholipid-cholesterol membrane model[4]. We have sought more direct evidence on lanthanum blocking of cell membrane calcium transport, and hoped in so doing to obtain additional information regarding calcium transport in general.

A solution (0·3 µl.) of ^{45}Ca, 0·5 mCi/ml. in 0·5 M KCl, was injected into squid axon longitudinally according to the method of Caldwell *et al.*[12]. The final concentration of the added calcium in the axoplasm was 0·3–0·5 mM. After the injection, the chamber in which the axon was suspended was reduced to 1 ml. and perfused with the experimental solutions at the rate of 0·8 ml./min. The perfusion solutions were collected every 3 min, dissolved in Bray's scintillation fluid, and counted in a Packard liquid scintillation counter. The axon was exposed to 1 M HCl at the end of the experiment, and counted in Bray's scintillation fluid after neutralization and addition of an equal aliquot of seawater. The external perfusion solution was: 425 mM NaCl, 10 mM KCl; 50 mM $MgCl_2$, 10 mM $CaCl_2$, 0·1 mM EDTA and 2 mM Tris (pH 7·8). In the calcium free solutions, $CaCl_2$ was replaced by $MgCl_2$.

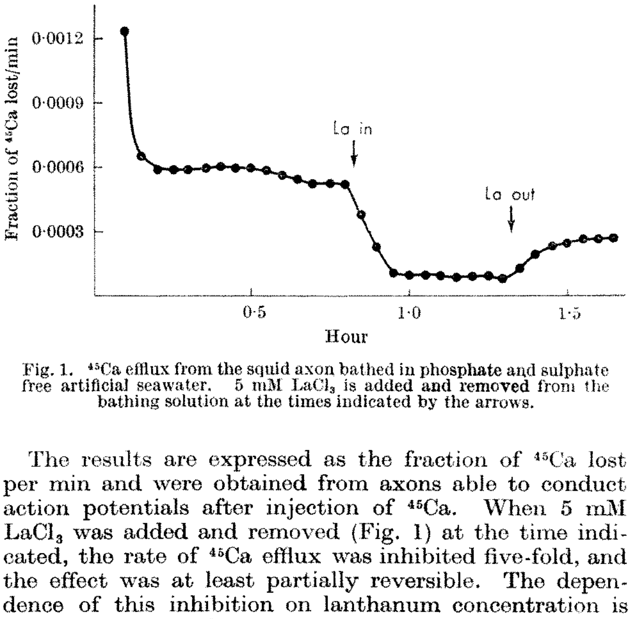

Fig. 1. ^{45}Ca efflux from the squid axon bathed in phosphate and sulphate free artificial seawater. 5 mM $LaCl_3$ is added and removed from the bathing solution at the times indicated by the arrows.

The results are expressed as the fraction of ^{45}Ca lost per min and were obtained from axons able to conduct action potentials after injection of ^{45}Ca. When 5 mM $LaCl_3$ was added and removed (Fig. 1) at the time indicated, the rate of ^{45}Ca efflux was inhibited five-fold, and the effect was at least partially reversible. The dependence of this inhibition on lanthanum concentration is shown in Fig. 2 and these results can be plotted according to the equation

$$\text{inhibition}/\text{inhibition}_{\max} = (\text{La})^e/[(\text{La})^e + K_D]$$

where $K_D = 0{\cdot}23$ mM and the maximum inhibition is 87 per cent.

Thus, in the presence of 10 mM external calcium, the half saturation value for the inhibition of calcium efflux by lanthanum is 0·23 mM. Removal of external calcium reduced this value to about 0·1 mM.

Lanthanum at a concentration of 5 mM rendered the axon inexcitable, after the action potentials first became of long duration and low amplitude. Only 0·1 mM $LaCl_3$ was sufficient to abolish spontaneous firing of the axon in calcium and magnesium free seawater and restore normal excitability.

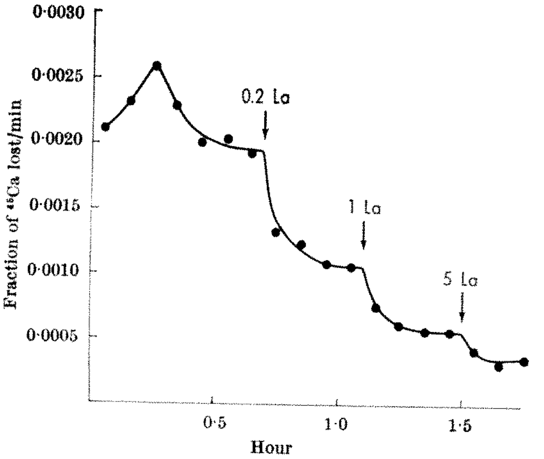

Fig. 2. ^{45}Ca efflux from the squid axon in the absence of LaCl$_3$ and presence of 0·2 mM, 1 mM and 5 mM LaCl$_3$. Concentrations of LaCl$_3$ are changed at the times indicated by the arrows.

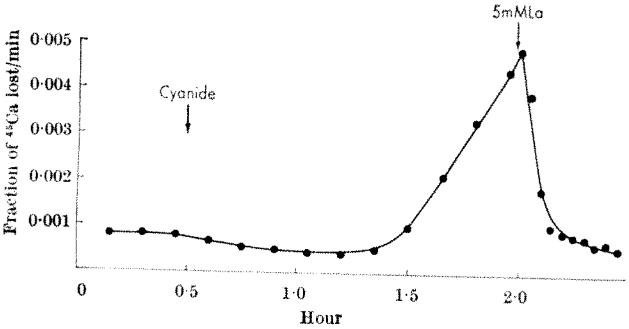

Fig. 3. ^{45}Ca efflux from the squid axon. At the first arrow 2 mM cyanide is included in the bathing solution. At the second arrow 5 mM LaCl$_3$ is also added.

Fig. 3 confirms the increase in ^{45}Ca efflux following 2 mM cyanide reported by Blaustein and Hodgkin[8], and shows, in addition, that this increased ^{45}Ca efflux is also inhibited by lanthanum.

Using the value of 0·41 mM calcium/kg axoplasm[8], the average efflux for axons of *Loligo pealeii* (average diameter 450 μm) was 0·25 ± 0·02 pmole cm^{-2} s^{-1} (ref. 10), in good agreement with values in the literature[8,13]. Of this efflux, 87 per cent could be inhibited by lanthanum ions, which seemed to compete with external calcium ions for binding sites on the cell membrane.

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Strychnine inhibits the Binding of Glycine to Rat Brain-Cortex Membrane

THE excitatory action of strychnine on the central nervous system results from its ability to antagonize the effect of synaptic inhibition[1]. Recently, evidence has accumulated for glycine as an inhibitory transmitter, especially at lower levels[2,3]. Because strychnine is capable of antagonizing the inhibitory effect of iontophoretically applied glycine[3], the contention is that it does not interfere with transmitter liberation, but interacts with receptors located on postsynaptic membranes, rendering them less sensitive to glycine-mediated inhibition. *In vitro* studies of the mechanism of action of strychnine, concerned chiefly with the active transport of glycine, have invariably yielded negative results[3,4].

We now report experiments concerning the binding of glycine to central nervous system membranes, and the effect of strychnine on it. The membranes used were obtained from dissected cerebral cortices of adult rats of Wistar descent. We followed essentially the procedure of Gray and Whittaker[5], except that the P$_1$ nuclear pellet was also washed once. The P$_2$ large-granule pellet is known to contain nerve-ending particles, with their postsynaptic membranes attached, mitochondria, myelin fragments and some other ill-defined membranes. We suspended it in 20 volumes of twice distilled water at 0° C for 15 min, and then centrifuged at 27,000*g* for 1 h to free it from synaptic vesicles, soluble enzymes and other materials enclosed in the particles and retain only the membrane components. The crude membrane pellet was finally resuspended in 5 ml. of 0·32 M sucrose.

Binding of glycine to the membranes was studied. Membrane suspension (0·1 ml.) was added to test tubes containing inorganic salts and buffer, with a composition similar to that in cerebrospinal fluid (final concentrations being 123 mM NaCl, 2·5 mM KCl, 0·93 mM CaCl$_2$, 0·86 mM MgCl$_2$, 20 mM Tris-HCl (*p*H 7·3), in a final volume of 2·0 ml.). Experimental tubes also contained the amounts of strychnine sulphate indicated in the figures. The reaction was started by adding 1-^{14}C-glycine (10 Ci/mole). Tubes were incubated at 37° C for the times shown in the figures. The reaction was stopped by filtering the tube contents through nitrocellulose membranes ('Millipore' type 'HA', 0·45 μm) and washing rapidly three times with 5 ml. of the indicated salt solution kept at 0° C. The filters with their quantitatively retained membranes and bound glycine were dried at 100° C, and counted in a conventional toluene based scintillator in a Nuclear

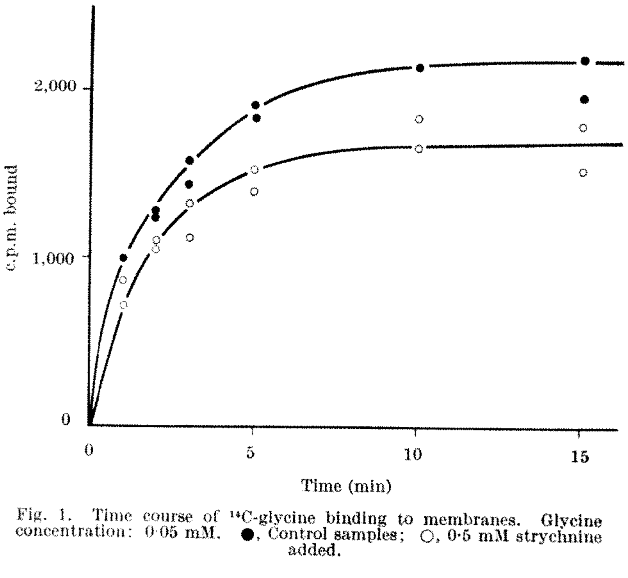

Fig. 1. Time course of ^{14}C-glycine binding to membranes. Glycine concentration: 0·05 mM. ●, Control samples; ○, 0·5 mM strychnine added.

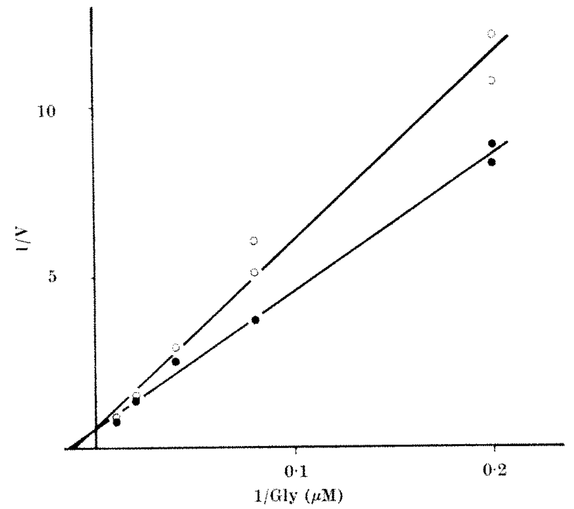

Fig. 2. Lineweaver–Burk plot of ^{14}C-glycine (specific activity 10 Ci/mole) binding to membranes. Reactions were stopped at 1 min of incubation. 1/V is (1/c.p.m.) × 10^3. ●, Control samples; ○, 0·5 mM in strychnine. All determinations were done in duplicate: when only one point appears at a given concentration it is because duplicates coincide.

Chicago scintillation spectrometer. Tubes with a final volume of 10 ml. and kept at 0° C were filtered immediately after adding the membrane suspension. These served as blanks and their radioactivity was subtracted from the experimental tubes.

The binding time-course (Fig. 1) is considerably faster than that observed in studies of glycine transport *in vitro*[4,6]. Initial velocities last only up to 1 min, and at 10 min equilibrium is reached. Strychnine inhibits the binding by about 25 per cent at this concentration. Raising the alkaloid concentration leads to progressively larger inhibitions, till at 10 mM (the greatest tested) it becomes 76 per cent. In some membrane preparations inhibitory effects have been detected at 2·5 μM strychnine, but adequate conditions for obtaining reproducible results at this low concentration have not yet been found. The dependence of initial velocities on glycine concentration (Fig. 2) gives a half-saturation value of 78 μM, which is much lower than that found for glycine transport in brain slices (440 μM) (ref. 6). These kinetic differences between our results and those dealing with transport suggest that both processes are of different nature. The type of inhibition, as seen in a double reciprocal plot, is strictly competitive. This was unexpected, because it may be considered from what we know that glycine binds not only to postsynaptic receptors but also to transport and to non-specific sites, and a more complex type of inhibition by strychnine was predicted. This finding may be interpreted in two ways. Either one of the binding sites greatly predominates over the other two, and strychnine competes with glycine for it, or second, more than one of the theoretical binding sites for glycine contribute appreciably, but all have very similar dissociation constants, on the one hand, for glycine (K_s), and on the other, for strychnine (K_i). The latter alternative seems less probable, but cannot be rejected with the available data.

High strychnine concentrations are generally applied *in vivo* to the cerebral cortex to obtain excitatory effects[8]. This lower sensitivity to the drug, relative to lower neural structures, may be an explanation of the fact that the *in vitro* effect described requires a strychnine concentration much higher than is necessary to suppress inhibition on spinal neurones[3]. It is also frequently the case that elicitation of drug effects *in vitro* need high concentrations[9]. Although the possibility of a less specific *in vitro* effect of strychnine cannot be wholly excluded, its competitive antagonism for glycine binding correlates well with previous physiological data[7], and suggests that this type of study may lead to a better insight into the molecular mechanisms of nervous inhibition and its modification by drugs.

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Theories of Milk Secretion: Evidence from the Electron Microscopic Examination of Milk

IN spite of study for over one hundred years, we still do not know how milk is secreted by the mammary cells[1]. One early theory of light microscopists was that the mechanism was holocrine, involving death and release of the whole cell as in sebaceous glands. Indeed, recent biochemical studies have shown that milk contains several intracellular components (such as citrate, enzymes and microsomes), although it is uncertain whether these are derived from secretory or duct cells or from phagocytic cells (neutrophil leucocytes and macrophages), which are known to migrate into milk from the blood and connective tissue. Later, the idea that whole cells are lost was rejected because the observed rate of mitosis was too small to account for the predicted rate of cell renewal. An alternative theory was that the process was apocrine—that only the apex of the cell became detached and that milk consists essentially of cell debris. Subsequent investigators considered this theory was not proven because what seemed in histological sections to be pieces of cytoplasm recently detached from cells could have been portions of neighbouring cells, the greater part of which lay out of the plane of section[2].

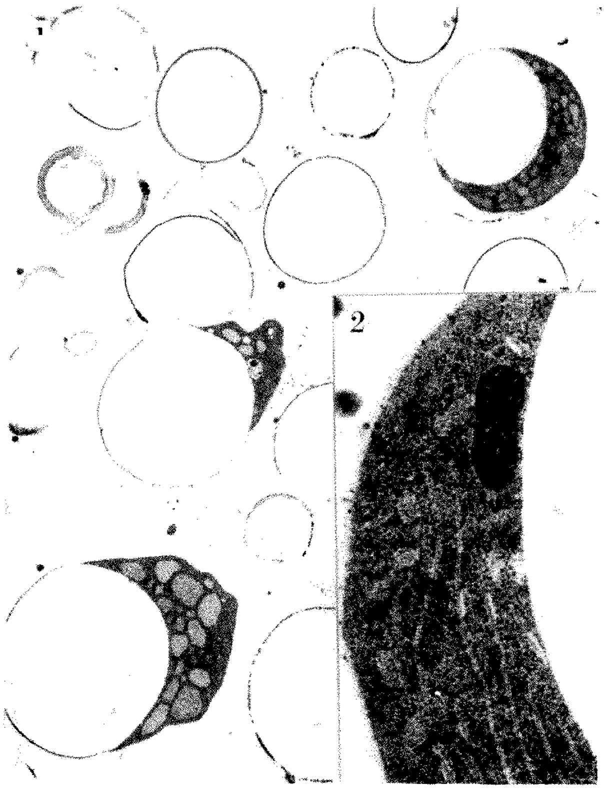
Mammary glands have now been examined with the electron microscope in several laboratories. Bargmann and Knoop[3] proposed that protein particles probably escape when Golgi vacuoles open at the surface and that fat globules are pinched off from the apices of secretory cells with cell membrane around them; holocrine and apocrine mechanisms were rejected. Two groups of workers[4,5], however, have revived the apocrine theory because they found crescents of cytoplasm within the plasmalemma (cell membrane) around some fat globules ("signets") free in the lumen of the alveolus but near the secretory cells. As with light microscopy, it is difficult to be sure that such signets are not connected to neighbouring cells; that they are not would be certain if signets were to be found in large ducts well away from secretory cells. Technically it is difficult to examine the contents of large ducts by electron microscopy. We thought it possible, however, that a few signets might still be present at the teat, that is, in the milk, which can be processed for electron microscopy. We now report the idea to be correct and that, as well as settling the controversy, the examination of milk confirms that an apocrine process is involved in milk secretion as classical histologists described it, because we find that what are generally described in the literature as epithelial cells in milk are pieces of cytoplasm devoid of a nucleus, which we believe also come from the secretory cells in the goat.

Fresh milk (2 ml.) from seven goats and a guinea-pig was mixed with an equal volume of 4 per cent glutaraldehyde and 2 per cent sucrose in 0·1 M phosphate buffer, *p*H 7·2. The mixture was spun at 900*g* for five minutes, producing a floating layer of cream and a pellet of denser protein and cells. The cream and protein pellets were treated separately with Palade's buffered osmium solution, embedded and sectioned by standard techniques. The middle layer contained only protein granules and was not examined in every case.

Fig. 1 shows that the cream contained a significant number of signets identical to those seen in alveoli by Helminen and Ericsson[4] and by Kurosumi *et al.*[5]. Both signets and other lipid globules were bounded by a unit (that is, cell) membrane; this indicates their cellular origin, for the lipid globules deep within the cytoplasm of the secretory cell do not have a bounding membrane, nor does that portion of the lipid globule of the signet adjacent to the cytoplasm. The signets contained rough endoplasmic reticulum, which was often considerably swollen, numerous free ribosomes (Figs. 1 and 2), well preserved mitochondria (Fig. 2) and occasionally membrane bound vesicles containing granules (Fig. 1). These vesicles are identical to those found in the Golgi region of the goat secretory cell (unpublished observations).

It is generally accepted that the unit membrane found around secreted lipid globules represents that part of the plasmalemma of the secretory cell which invests the lipid globule as it is extruded through the cell surface. When the extrusion is almost complete, the globule will be attached to the cell by a narrow neck of cytoplasm. If this neck separates immediately adjacent to the lipid, a globule with no cytoplasm will be produced, as is mostly found in the alveoli and in milk (Fig. 1). If, however, the separation occurs closer to the cytoplasm, then a variable amount of secretory cell cytoplasm will be included and will produce a signet. Signets have been found in all specimens of milk that we have examined, both from normal animals and from those treated with oxytocin (0·1 unit intravenously). A rough estimate suggests that from 1 to 5 per cent of the lipid globules seen were signets.

Examination of pellets from the bottom of the tube showed that, for goat milk, the greater part consisted of closely packed protein granules identical to those seen in Golgi vacuoles in the cell and in the lumina of the alveoli (Fig. 3). As expected, the lowest layer also contained a few neutrophil leucocytes, monocytes and macrophages and an occasional red blood cell, but, unexpectedly, 85 per cent of the total objects seen in any field were cytoplasmic fragments, fewer than 0·1 per cent of which contained nuclei (Fig. 3). Although examination of cows' milk smears by light microscopy for the identification of bacteria and cells is routine in many animal health laboratories, it is surprising that only Paape and Tucker[6] have reported that unidentified cell fragments outnumber leucocytes in normal milk, and that the superior resolution of the electron microscope has not been used to examine normal or diseased milk. In the goat the size of the cytoplasmic fragments varied between 5 and 25 μm; on average they were larger than the neutrophils (Fig. 3). Small lipid droplets were also found in them (Figs. 5 and 6) together with vesicles containing protein granules identical to those in secretory cells. The major difference in the appearance of the fragments and that of alveolar secretory cells was that the endoplasmic reticulum was swollen to a variable degree (Figs. 3–6). Apart from the white blood cells and macrophages, no true cells containing a nucleus have been recognized. Like the signets, the fragments were a constant feature of all goat milk so far examined. The number was similar in the first and last milk removed at a milking but was increased after injecting oxytocin. Again, no nuclei were seen in any of the fragments.



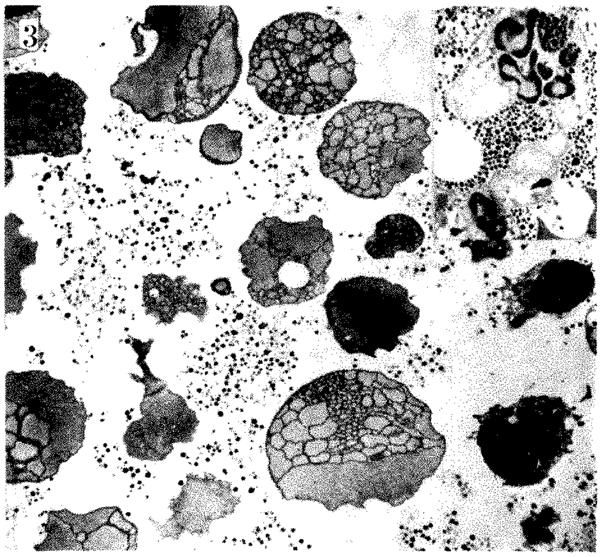
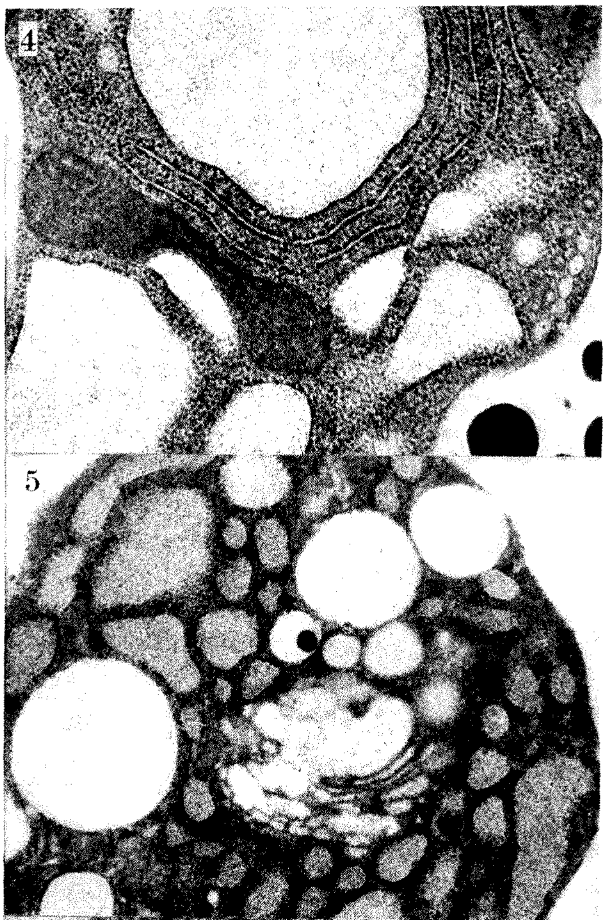


Fig. 1. Micrograph of the floating layer (cream) from goat milk after fixation and centrifugation. It consists largely of lipid globules surrounded by a unit membrane (not resolved at this magnification). Three of the globules shown also have cytoplasmic material on one side and represent the "signets" referred to in the text. (× 4,800.)

Fig. 2. Higher magnification of the cytoplasmic material present on some lipid globules. A mitochondrion, free ribosomes and cisternae of the rough endoplasmic reticulum can be seen. (× 28,000.)

Fig. 3. Micrograph of the bottom layer of the pellet from goat milk after fixation and centrifugation. The small black dots between the cytoplasmic fragments are the milk protein granules. Two white blood cells (neutrophils) are present on the right hand side of the micrograph. Most of the area of the cytoplasmic fragments is taken up with swollen endoplasmic reticulum. Inset is a macrophage-like cell at the same magnification (× 2,200).

These cell fragments could originate only from phagocytic cells or from the mammary duct and secretory cells. They do not seem to be degenerating or effete mammary cells, which might be expected to contain a nucleus or its remains and cytosegrosomes, which are seen in cells of involuting mammary glands[4], but not a well preserved cell membrane. Nor do they seem to be duct or teat lining cells which would be expected to contain a nucleus, tonofilaments, desmosomes and, if from the teat, keratin. Further, they do not seem to be portions of leucocytes or macrophages because their cytoplasm does not contain neutrophil granules or phagocytotic vesicles. In human colostrum, macrophages have been shown to be ingesting fat and protein granules[7] and, in our preparations of milk, both macrophages and neutrophils contain ingested fat and protein particles which were surrounded by the membrane of the phagocytotic vesicle (Fig. 7), whereas no such membrane was seen around fat droplets in the signets or in the cell fragments (Figs. 1, 2, 4 and 6).



Figs. 4 and 5. Details of two cytoplasmic fragments from different batches of milk. The endoplasmic reticulum is swollen to differing degrees. Fig. 4 contains a central area of stacked vesicles interpreted as part of the Golgi complex immediately beneath which lies a vesicle containing a milk protein granule. A lipid droplet is present to the right of the Golgi area. Fig. 5 shows a mitochondrial profile and both swollen and normal endoplasmic reticulum. Fig. 4, × 22,000; Fig. 5, × 30,000.

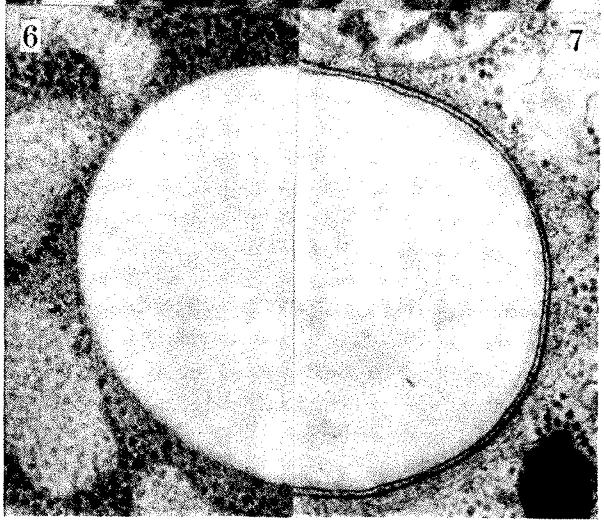


Fig. 6. Part of a lipid droplet in a cytoplasmic fragment. There is no visible membrane bounding the lipid droplet. (× 65,000.)

Fig. 7. Part of a lipid droplet in a leucocyte bounded by a membrane which is in turn surrounded by the membrane of the digestive vacuole. (× 42,500.)

The only other possible source of the fragments is the secretory cells of the alveoli and smallest ducts. Further evidence in favour of this source is that they closely resemble the appearance of the secretory cells, particularly in possessing fat and protein particles, abundant rough endoplasmic reticulum, a hypertrophied Golgi body and intact mitochondria. Admittedly, the endoplasmic reticulum of the milk cell fragments was swollen, but we have also seen such swelling in goat mammary tissue after oxytocin treatment. Our conclusion is therefore that, like the signets, the fragments are part of the secretory cells lost in a classical apocrine manner. The increase in their number seen after an injection of oxytocin may be significant. It seems possible that, in addition to the normal release of large fat globules and the possibly less common pinching off of signets, a cell that is swollen with secretory products well above the level of the tight junction with the next cell might lose part of its apex under the influence of external forces such as myoepithelial cell contraction. An attractive feature of this hypothesis is that the mechanism of milk secretion may vary according to the degree of fullness of individual alveoli.

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Cell Division in the Early Growth and Morphogenesis of the Chick Limb

The confusion concerning the relationship between growth and form in animal development is quite deep and widespread and possibly stems from the title of D'Arcy Thompson's great book[1], together with the botanists' emphasis on localized growth zones such as the apical meristem. Our attention was drawn to this during our studies on budding in hydra[2], where it had always been assumed that formation of a new hydra by budding was partly the result of localized growth at the base of the bud. From our experiments it now seems that bud elongation does not require cell growth and division but is a morphogenetic process involving cell movement and changes in cell contact. This led us to question whether cell growth and division ever determine in a significant manner the form, as distinct from size, of animal tissues and we have had difficulty in finding examples where this is obviously the case. Orientated cell divisions in early development contribute to the morphogenesis of the blastula[3] and are almost certainly important for the determination of form in mosaic embryos. In these cases it is the plane of cell division and not growth that is important. The growth of certain insect structures involves orientated cell divisions and is one of the very few cases where growth seems to determine form[4]. Form seems to be determined by the spatial relationship between the cells which may be largely determined by cell movement and cell contact[3].

The early development of the chick limb[5,6] is characterized by rapid outgrowth and elongation which transform the initial, simple, beehive shape into an elongated structure that is slightly flattened dorso-ventrally. The bud at this stage is made up of undifferentiated loose mesenchyme with an epidermal covering. A prominent ectodermal ridge, most fully developed at the distal end, runs antero-posteriorly. During the early stages of elongation, stages 17 (Hamburger and Hamilton) to about 20, the presumptive regions of the wing along the proximo-distal axis are specified in a proximo-distal sequence[1] (Fig 1), that is, shoulder region, then humerus, then radius and ulna followed by the digits. The presumptive maps up to stage 19 are congruent. This specification of presumptive regions does not imply that regulation is impossible, and considerable regulation is possible[5,6] up to about stage 23 or 24. There are three main questions relating to the growth of the early limb bud: (1) what provides the force for the early outgrowth? (2) what determines the overall paddle shape of the early limb? and (3) what is the relation between the growth of the early limb and the proximo-distal specification of the regions[8]? The last question is raised by the congruence of early presumptive regions and by the claim of Saunders[7], based on carbon making experiments, that only a narrow region at the tip is relevant to increase in length. Amprino[5], too, has claimed that during early stages there is a greater rate of proliferation in the distal mesoderm compared with the proximal. Ede and Law[9] have suggested on the basis of computer simulation that a proximo-distal gradient in growth rate, together with distal movement of the cells, may provide for limb elongation. Of course, distal movement of cells alone could in principle lead to distal elongation. Because adequate quantitative data on mitosis during limb bud outgrowth were unavailable we have investigated it as a first step towards answering the above questions.

White Leghorn embryos were incubated at 38° C and fixed in 5 per cent TCA (Jurand[10]) and double embedded[11]. Sections were cut at 7 μm parallel to the long axis of the limb and at right angles to the dorso-ventral plane. They were stained in haematoxylin and eosin.

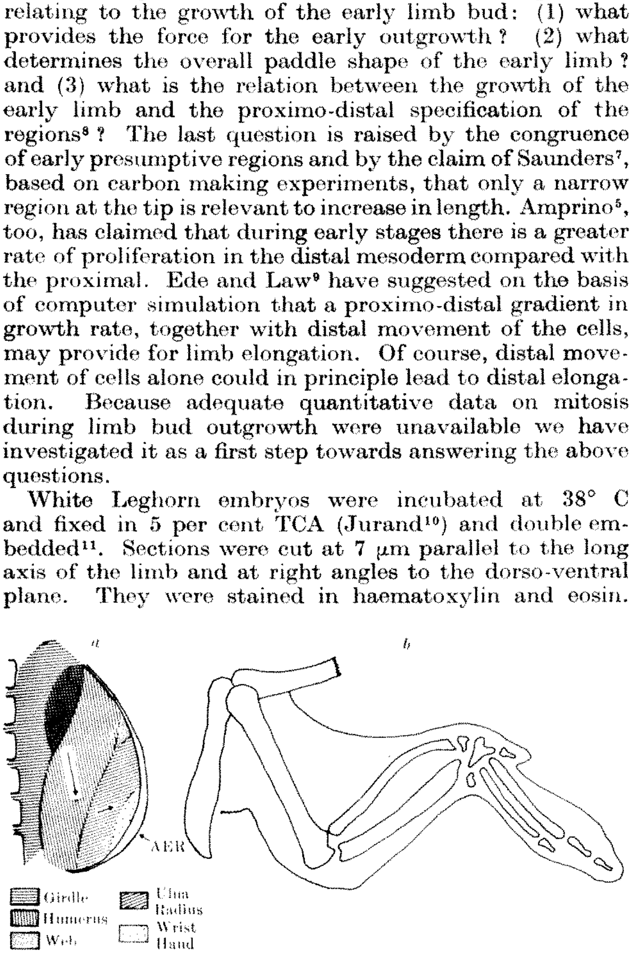

Fig. 1. *a*, Limb bud at stage 19 (after Saunders[7]) to show presumptive regions. AER, Apical ectodermal ridge; *b*, the wing after about 10 days' incubation.

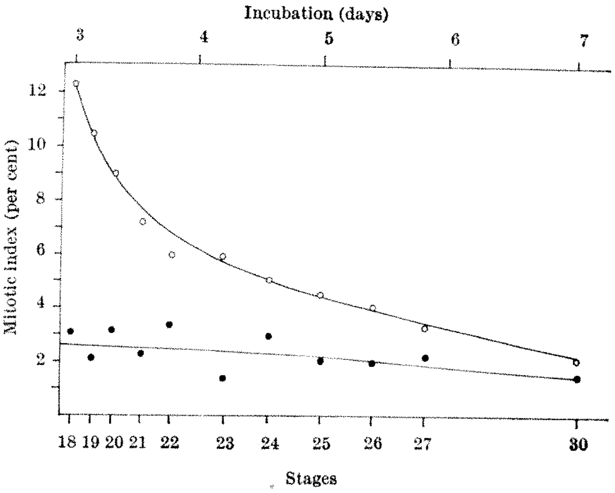

Fig. 2. Overall mitotic index of mesenchyme (○) and ectoderm (●) at different stages.

For the counting of mitoses in the mesenchyme an approximately medial section was chosen together with one section located ten to fifteen sections away on either side. Every cell and every mitosis in these sections were counted under oil immersion, by dividing the section into strips about 150 μm wide and containing not less than 200 cells, and at later stages not less than 500 cells.

The overall mitotic index of the mesenchyme is initially very high, about 10 per cent at stage 18, but drops steadily to about 2 per cent three days later, at stage 30 (Fig. 2). This striking drop in mitotic index could be the result both of an increase in the cell cycle time in relation to that of mitosis and of a reduction in the number of cells dividing. We cannot distinguish the relative contributions of the two factors without a much more elaborate analysis, but we are inclined to suggest that the first is probably more important in early stages, for Searls[12] found that about six hours after injection of tritiated thymidine at stage 22 all nuclei were labelled, but it required ten hours at stage 24. Whatever the cause, a reduction in the mitotic index would be correlated with a reduction in growth rate of the limb if cell division were the main factor providing for its growth. Schmalhausen's[13] data on the increase in mass of the limb show that the growth rate does decrease during early stages. Studies of multiplicative growth of chick wings in terms of DNA content are only available from 5–12 days[14], that is, after stage 26, during which the growth rate is constant. We have, however, observed a small drop in the mitotic index between the fifth and the seventh day of incubation. It is of interest that Nowinski and Yushok estimated the mitotic index in 6–7 day wings (stage 30) at 2·5 per cent, which is in reasonable correspondence with our data.

The overall mitotic index of the ectoderm is also shown in Fig. 2, and fluctuates around 2 per cent. The smaller mitotic index of the ectoderm compared with the mesenchyme may partly be anticipated on the grounds of the volume/surface relationship of the extending bud.

The regional pattern of mitotic indices within the limb bud mesenchyme at various stages is summarized in Fig. 3. Between 18 and 23 there is no significant difference ($P < 0{\cdot}01$) in the mitotic index of proximal and distal regions (as determined by χ^2), but from stages 24 to 30 there is a significant decrease in the mitotic index in the proximal regions compared with the distal regions. The situation during stages 21 to 24 is a little more complicated, for some regions of very high mitotic index were found in localized patches, in the elbow region at stage 21 and in the wrist region at stage 23. The constancy or significance of such localized regions of high mitotic index is not known. Mitoses in the ectoderm seem more or less

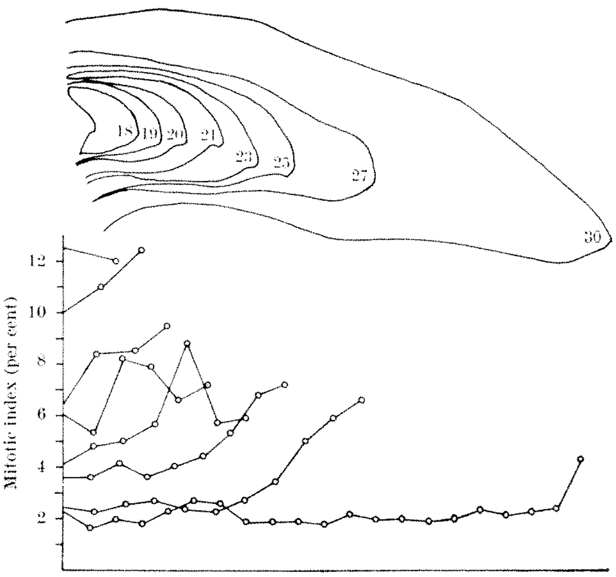

Fig. 3. The axial distribution of mitotic index at different stages. The upper drawings give the outline of the limb at the appropriate stages.

evenly distributed, though occasionally a patch of high mitotic index, apparently associated with synchrony of division, was seen at the tip of the apical ridge.

The orientation of mitoses within the mesenchyme was examined at stages 20 and 24 to look for a preferred plane of cleavage. The scoring of the planes is difficult and, apart from a suggestion that there are fewer divisions in the direction of the long axis, no differences could be claimed.

The results can be summarized by saying that the fall in mitotic index, as the bud grows, occurs more rapidly in the proximal region after stage 24, so that a proximo-distal increase in mitotic index becomes established. Because there is considerable limb bud elongation before stage 24 a gradient in cell division is unlikely to be important in providing a mechanism, although it may have a role in later stages. We would suggest instead that the gradient in cell divisions from stage 24 onwards is related to the regional differences in the limb as can be seen from Fig. 1. The presumptive humerus region is much longer than the presumptive radius and ulna and the presumptive wrist and hand, although by stage 30 the latter are equal in length or even longer than the humerus. This requires that they grow faster. It is of interest that the time of appearance of the gradient is approximately that at which determination of the regions along the proximo-distal axis becomes fixed and at which one would expect them to display different properties. The absence of a gradient in cell division before stage 20 implies that cells are probably changing their presumptive fate in this period and behave according to their positional information[8]. In considering limb bud elongation one should distinguish between the forces producing the outgrowth and those factors determining its form. The forces involved are unknown but may be provided both by the increasing cell volume and the secretion of intercellular matrix. The latter seems particularly likely when, after stage 26 following condensation, the chondroblasts are forced apart by the secretion of matrix. The direction of separation is mainly along the long axis and this could contribute to limb elongation. As regards the overall form of the limb, particularly at earlier stages, we would suggest that it is determined by the ectodermal sheet, whose form is in turn determined by the nature of its intercellular contacts. Our work on sea urchin morphogenesis has suggested that the form of ectodermal sheets may be related to the degree of contact between the cells[3]. More specifically, a large degree of contact was always associated with an increased curvature of the sheet. A similar situation seems to occur in the limb bud, the apical ridge being the region of increased cellular contact and increased curvature. Electron microscopy of limb buds confirms the idea that the form is determined by the ectoderm, for it appears as a discrete structural layer in contrast to the rather spongy mesenchyme.

Finally, it is necessary to consider the factors involved in the reduction of the initially very high mitotic index. It will be important to know whether this is part of a temporal programme of the limb bud cells or is determined by the cells' positional information and the measurement of limb bud length.

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Viral Components in the Genetic Background?

EXTRACHROMOSOMAL hereditary elements may be very much more important than present genetic convention supposes, and Gruneberg's[1] hypothesis that extranuclear entities are responsible for differentiation in skeletal patterns between sublines of mice is intriguing. But the rejection of the alternative view that changes in nuclear genes are responsible for this effect is supported by an argument that is not wholly convincing.

The crucial difficulty in explaining this differentiation by mutation of nuclear genes is that a mutation rate per gamete of 10^{-2} is inferred from data on skeletal variants and is incompatible with rates of mutation of the order of 10^{-6} observed by Russell and others. We should note, however, that Russell's data are for specific loci while Gruneberg's data are for skeletal characters. Some, perhaps all, of these may be regarded as quantitative or quasi-quantitative, and we may therefore ask how many loci may be involved in determining such characters and what estimates of mutation rates are available for such loci.

As far as I am aware, no systematic attempt has been made to determine directly, by genetic analysis, the number of loci (polygenes) governing a quantitative character, although Thoday[2] has shown that they can be characterized and mapped with suitable methods. Davies[3] has mapped loci influencing sternopleural chaeta number in a series of lines of *Drosophila melanogaster* in which selection on this character had taken place. His findings indicate that at least sixteen loci must be concerned with this character, and if other work is taken into consideration the number rises to twenty or possibly more. There is no reason why sternopleural bristle number should be atypical of quantitative characters in general and it seems likely that the skeletal characters used by Gruneberg will have similar genetic bases. If this is so, clearly the "per locus" mutation rate might be one twentieth of the figure given in the paper, or about 5 × 10^4. Further, because the estimate of twenty loci may be appreciably smaller than the average number of operative loci per character, the true value of the mutation rate may be smaller still.

The mode of action of such genes acting on one character may be very different from locus to locus, as Spickett[4] has shown. He analysed the effect of "high" alleles at two loci affecting sternopleural chaeta number in *Drosophila* and showed that one worked by increasing fly size and therefore bristle number generally, while the other increased the number of bristles in the ventral portion of the sternopleural region.

In a few cases it has proved possible to connect genes affecting quantitative characters more directly with their immediate products. Ashton *et al.*[5] have shown that about 10 per cent of the genetic variation of milk yield in given herds can be ascribed to the polymorphism for two transferrin alleles giving electrophoretically distinguishable products. A milk polygene is a transferrin major gene. In my laboratory Birley has been able to show that a similar situation exists with respect to rate of development and polymorphism at the esterase-6 locus in *Drosophila melanogaster*. In his material about 19 per cent of the phenotypic variance of speed of development is attributable to this polymorphism, and the esterase major gene is a developmental rate polygene. Data of this sort make it easier to accommodate polygenes within the framework of knowledge of gene action.

Next we have to consider estimates of mutation rates. Classically these have depended largely on methods like that of scoring sex-linked lethal mutations in *Drosophila* and coat colour mutations in mice and they lead to estimates of mutation rate of about 10^{-6}/locus. Other workers, however, have produced evidence which suggests that this figure may be too small. Whitfield *et al.*[6] concluded that many mutations giving an altered protein in *Salmonella* were probably not picked up by the selective techniques used so often in mutation work. Mukai[7] found that the rate of mutation of viability genes was twenty times greater than that for mutations to lethality in the same chromosomes. Confirmation of these data is desirable but, at face value, they suggest that estimates of mutation rate could therefore be appreciably smaller than the real values. The discrepancy between the two types of estimate is resolved by recognizing that the first group is scoring only mutations of large effect while in the second group, with a greater resolving power, more mutations of smaller effect are picked up and greater values for mutation rate are therefore obtained. On the very reasonable assumption that, on average, a locus can mutate in many more ways to produce slightly altered function than to produce drastically altered function, it may be concluded that the "real" rate of mutation in which all types of mutational change are considered as summed together may be one order of magnitude greater than that indicated by coat colour and lethal studies or, say, 10^{-5}.

Now the genetic situation used by Mukai was that of isogenic chromosomes in which spontaneous viability mutations were accumulated and scored. It is thus to some extent comparable with the inbred and highly homozygous material used by Gruneberg, in which quantitative morphological characters were scored. It is not unreasonable, therefore, to suppose that the resolving power of Gruneberg's system may be more similar to that of Mukai than it is to the systems using lethals and coat colour genes. If this is the case the maximum observed mutation rate "per locus" of 5×10^{-4} should be compared with an expected value of 10^{-5}. In fact, Gruneberg states that the number of mutations in the mouse lines may be overestimated; taking his lower limit the observed mutation rate becomes $2{\cdot}16 \times 10^{-4}$. The agreement between observed and expected mutation rates, calculated in the way I suggest, cannot yet reasonably exclude the idea that differentiation in inbred mice sublines takes place by fixation of nuclear gene mutations.

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Migrating Marsupials and Drifting Continents

Martin[1] has discussed the problem of the present distribution of marsupials, confined to the Australian region and to South America (with a little comparatively recent immigration into North America). He concluded that marsupials could not have entered Australia from either Asia or Antarctica and suggested instead that they had evolved in a land mass originally situated in the central Pacific over the position of the Darwin Rise, the activity of which had split the land mass in the early Jurassic. One portion then drifted south-west, forming part of New Guinea and the now sunken Coral Sea Platform, so delivering its cargo of marsupials to Australia. The other half had drifted north-east to become incorporated in North America, its marsupials then colonizing that continent and eventually spreading to South America.

Martin's theory seems to be at such fundamental variance with the bulk of the present picture of continental movements and directions of sea-floor spreading that it could only be justifiable if it were the sole reasonable explanation of the known facts of palaeozoogeography. How the ancestors of the marsupials arrived in Martin's isolated mid-Pacific land mass is not the least of the problems involved in his theory, and I believe that other, more simple, theories explain the known facts at least as adequately.

As is well known, the problem peculiar to Australia is the presence there of marsupials but not of placentals. It has been commonly accepted that both groups appeared at approximately the same time. Acceptance of this view in turn implied that any easy route of access to Australia would have been traversed by both groups. Because this had not taken place, the implication was that there had never, since the evolution of the two groups, been a continuous land bridge between Australia and the rest of the world. Instead, the geographical character of the area had always been broadly as it is today, and the spread of both placentals and marsupials had been hindered by the existence of a number of sea barriers of varying width. The chances of either group making the complete passage from Asia to Australia were small; by chance, the marsupials had succeeded and the placentals had not.

The many discussions of the best positions through which to draw a line to demarcate the Australian zoogeographic region from the Oriental zoogeographic region (Wallace's Line, Weber's Line, Lydekker's Line, and so on) have stimulated much study of the faunas of the islands lying between Malaya and Australia. Nevertheless, it does not seem to have been realized that these faunas provide strong evidence against the view that marsupials spread along this chain of islands, followed at a later date by the placentals. If this had taken place, one would have expected the marsupials to have colonized the majority of these islands, and to have undergone some adaptive radiation there before the placentals arrived. Even if a variety of placentals had later colonized the islands, and even though they in general succeed when in competition with marsupials, it would have been surprising if they had completely exterminated every species of marsupial from nearly every island. Because few placentals have in fact succeeded in spreading to the islands east and north of the limits of the Sunda (Asian) continental shelf, it would be all the more expected that a variety of marsupials would still be found in many of the islands, particularly the northern fringe of islands, such as the Philippines. This, however, is not the case. In fact, the faunal picture is not of an existing marsupial fauna in the islands being progressively replaced from the west by invading placentals. Instead, it is of an archipelago, originally lacking any type of mammal, which is still being colonized—by placentals from the west and by marsupials from the east. Thus Darlington[2] refers to this area as a zone of subtraction and transition, and stated that Wallace's Line "separates the nearly full-scale Oriental fauna from a strikingly depauperate fauna". Darlington believes that the area has been colonized by vertebrates rather recently, and that more Oriental (that is, placental) groups than Australian (that is, marsupial) groups have extended into the area—a surprising fact if the marsupials had migrated through the region before the placentals. Similarly, Simpson[3] states: "From Australia marsupials have spread with a completely regular pattern of progressive attenuation as far as the Solomons to the north-east, Talaut to the north, and Celebes to the west." (Fig. 1.)

The clear implication of all the preceding facts is that marsupials did not reach Australia from Asia, and in this I agree with Martin.

In any discussion of faunal migrations in the late Mesozoic and early Tertiary, one of the greatest gaps in our knowledge is our almost complete ignorance of the reptile and mammal faunas of Africa through this time. A few large dinosaurs were found in the earlier part of this century, in the Upper Cretaceous of South Africa. These deposits have not yet, however, been re-examined with the care necessary to reveal the remains of the tiny early mammals, and it cannot therefore be assumed that they were absent from Africa. The earliest known mammals, *Erythrotherium* and *Megazostrodon*, were found in the Upper Triassic of southern Africa[4,5] and a single Upper Jurassic mammal, probably a pantothere, has been found in Tanzania[6]. The presence of Cretaceous dinosaurs in Africa suggests that there was still, from time to time at least, land connexions between that continent and Laurasia, where there was a varied dinosaur fauna (though it is also possible that these Cretaceous dinosaurs were merely the descendants of dinosaurs known to have existed in Africa in the Jurassic). At the very least, there is no evidence to rule out the possibility that marsupials were able to enter Africa some time during the Cretaceous. As in other continents, they would later have become extinct there because of competition from placentals in the early Tertiary.

If early mammals lived in Africa, the problem of their access to Australia depends on the precise times of origin of the marsupials and of the placentals, and of the time at which a land connexion to Australia was severed.

As has already been noted, a direct continuous land access-route to Australia will not explain the absence of placentals from that continent if these originated before, or at the same time as, the marsupials. But there is no evidence to make such an assumption necessary. The precise time of origin is unknown for either group. All

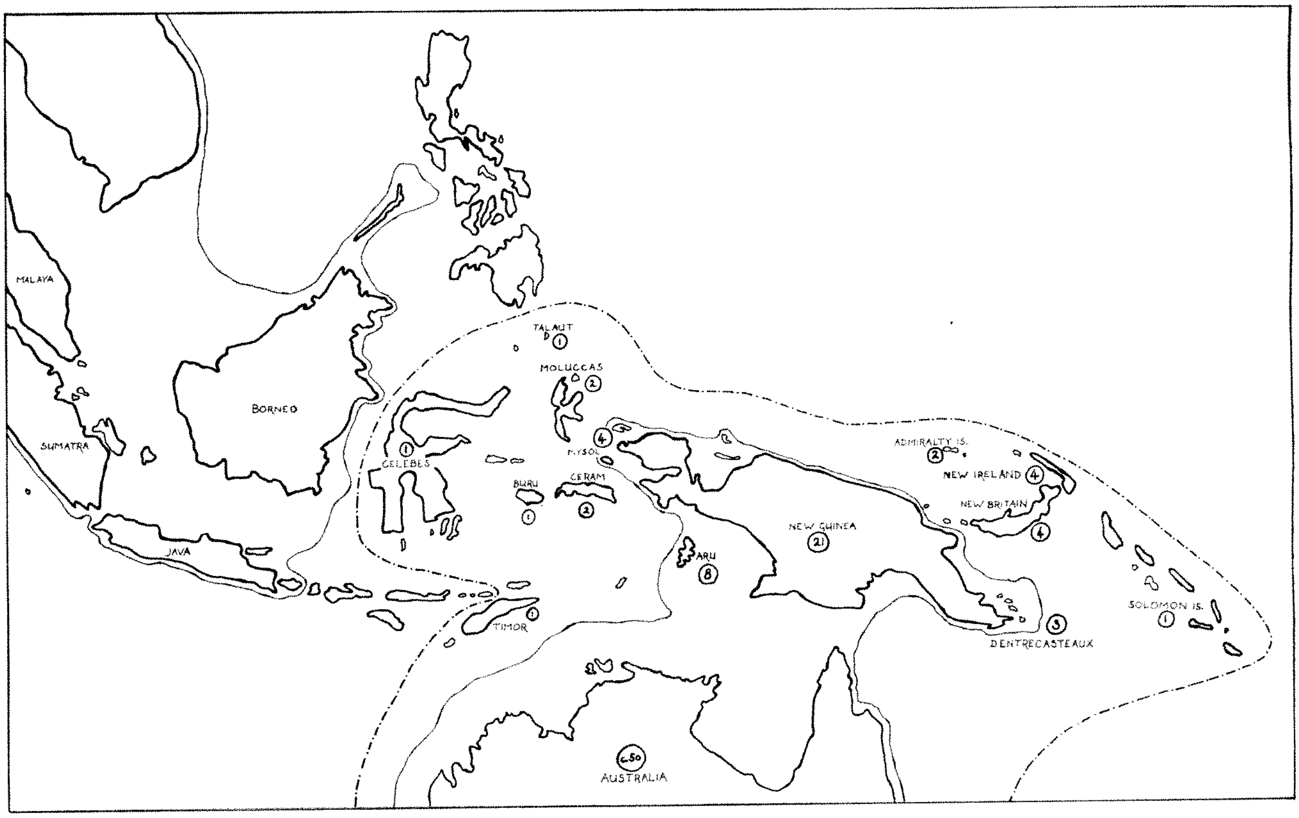

Fig. 1. **Islands of the Australasian region.** Figures indicate number of marsupial genera, after Simpson[3]. ———, Continental shelf (200 m contour); — · — · —, limit of marsupial distribution.

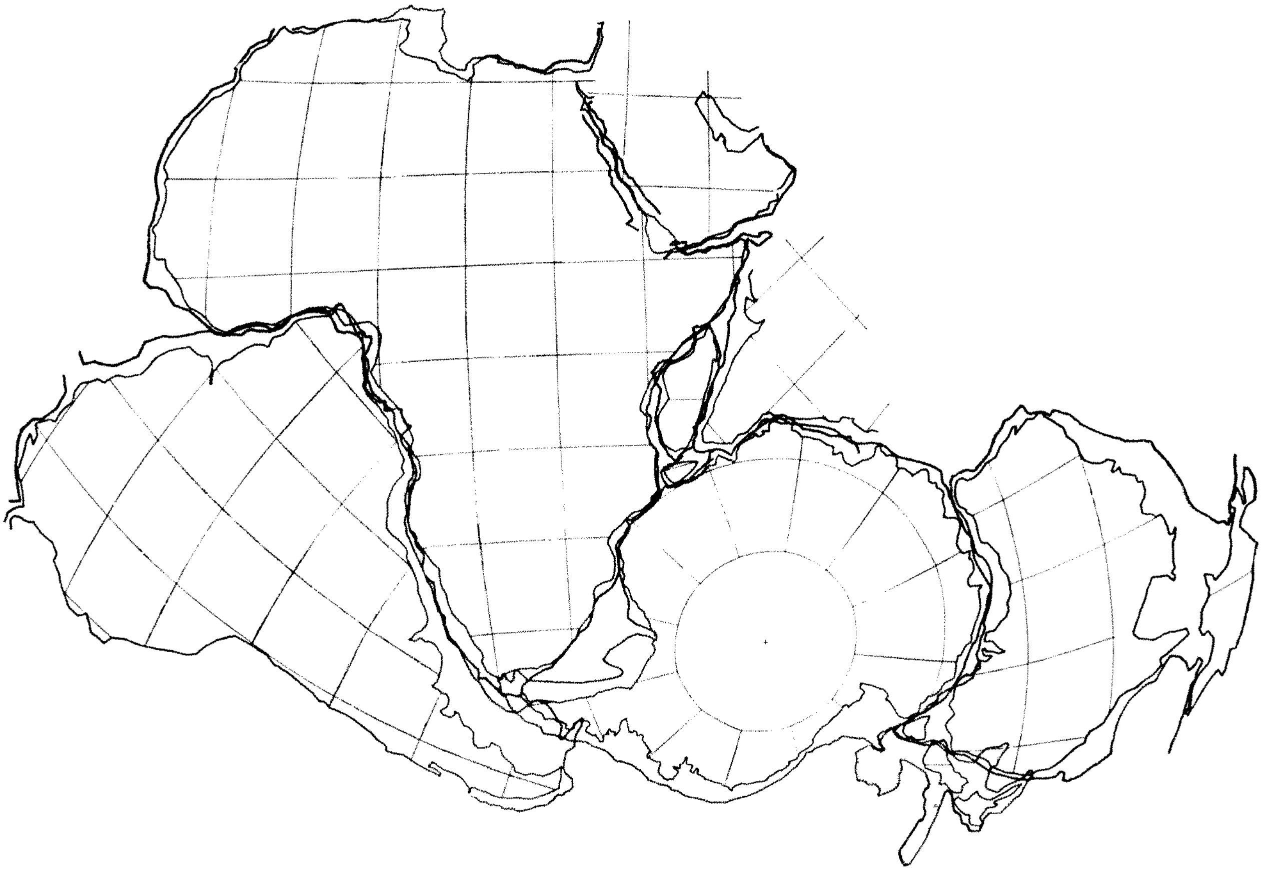

Fig. 2. The fit of the southern continents, from Smith and Hallam[10].

that is known is that members of both groups, identified by their different tooth patterns, were in existence in the Albian (Middle Cretaceous) of North America[7,8]. There is no reason here to assume that both groups had an equally long earlier history. Another possible reason for such an assumption would be the belief that the two groups were divergently specialized descendants of a common oviparous ancestral stock. In fact, as Lillegraven[9] has shown, it is far more reasonable to believe that the common ancestral stock was already essentially marsupial, and that the placentals are therefore specialized marsupials. He states: "It is my opinion that if it were possible to study living specimens of the ancestral therian stock, most observers would not hesitate to assign them to the infraclass Metatheria, order Marsupialia".

The few relevant facts, then, suggest that marsupials were in existence before the placentals. If so, the existence of Mid-Cretaceous placentals in turn suggests that the marsupials had differentiated by the Lower Cretaceous at the latest, having evolved from the Jurassic pantotheres[5]. If it is also accepted that they may have been able to spread to Africa at that time, the problem of deciding whether they could have spread from there to Australia is reduced to that of determining the time by which land access to that continent had been broken. In their discussion of the fit and break-up of the southern continents, Smith and Hallam[10] place Antarctica between the eastern side of Africa and the southern side of Australia (Fig. 2), providing a simple land route between these two continents. Discussing the time of break-up of Gondwanaland and of dispersal of the resulting continents, they state: "Palaeozoogeographic data on the distribution of terrestrial vertebrates and neritic molluscs suggest that widespread land connexions and negligible seaways existed between most of the fragments of Gondwanaland until well into the Cretaceous period." They conclude that, although much of the initial rifting (that is, small displacements of up to 100 km) probably occurred in Jurassic and Lower Cretaceous times, much of the dispersal occurred in Upper Cretaceous and Tertiary times. Smith and Hallam note the significance here of the syntheses of palaeomagnetic data by Briden[11] and McElhinny[12], both of which indicate that the divergence of their polar wandering curves shows significant dispersal of the Gondwanaland continents to have begun in the Middle Cretaceous. All this evidence, therefore, permits the suggestion that marsupials could have migrated from Africa to Australia via Antarctica in the Lower Cretaceous. The present climate of Antarctica is in itself, of course, a sufficient explanation of the present lack of marsupials in that continent and of our lack of any record of their presence there in the Cretaceous.

To summarize, then, it is likely that marsupial mammals were in existence before the placentals, which may have evolved from them later. It is at least possible that the marsupials were able to migrate throughout Africa, Antarctica and Australia before the Antarctica–Australia land mass separated from Africa, and that this in turn took place before the placentals reached Antarctica–Australia.

My theory, like Martin's, suggests the presence of marsupials in lands where they are unknown at present and unrecorded in the past. Because this is true of both of Australia's nearest neighbouring continents, such suggestions are an inevitable part of any theory of the origin of Australian marsupials, unless there were originally a direct connexion between Australia and South America. My theory, unlike Martin's, places these unknown marsupials on lands which are known to have existed, and whose movements do not conflict with the generally accepted theories of the directions of movement of the continents, and of sea-floor spreading, during the late Mesozoic and Tertiary.

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Resistance to *Claviceps purpurea* in Spring and Durum Wheat

The ergot fungus, *Claviceps purpurea* (Fr.) Tul., is common on cereals and grasses throughout the temperate regions of North America[1,2]. It is often severe on rye (*Secale cereale* L.) and durum wheat (*Triticum durum* Desf.) and in recent years it has also been troublesome on Triticale (*Triticum* sp. × *S. cereale*) and on male sterile cultivars and lines used in the development of hybrid spring wheat (*Triticum aestivum* L.) at the University of Manitoba. Field observations in the past have indicated that some cultivars of either spring or durum wheat are more susceptible than others[3], but experimental evidence of resistance has not been reported. We therefore investigated the variability of the infection reaction to *C. purpurea* in the cultivated cereals, aiming to find possible sources of resistance for use in the Triticale and hybrid wheat breeding programmes.

During these investigations two cultivars, one of spring wheat and one of durum wheat, were found to possess a higher resistance to ergot. These two cultivars were tested more extensively, along with two susceptible ones, and the results of the tests are the subject of this communication. A more detailed account of our studies, dealing with the selection and virulence of isolates and the testing procedures as well as the variability of the infection reaction in cultivated cereals, will be published elsewhere.

Ergot sclerotia were collected from the spring wheat cultivar "Manitou" and from the Triticale cultivar "Rosner". Two isolates from each cultivar were obtained by culturing surface-sterilized sclerotial pieces on acid malt agar. Conidia were increased as required in liquid shake culture[4]. The mycelium was removed by filtration and the spore density was adjusted to approximately 10^4 conidia/ml. with sterile distilled water. Plants of the wheat cultivars listed in Table 1 were grown in the greenhouse and inoculated two days before anthesis. Ten florets per head were inoculated by hypodermic syringe, injecting 0·02 ml. of the spore suspension into each floret. At least five heads of each cultivar were inoculated with each of the four ergot isolates and immediately covered with a small glassine envelope. The experiments were repeated four times for all cultivars, except "Carleton", which was tested twice. The inoculated florets were rated for the amount of honeydew produced ten days after inoculations. After twenty-one days, the number of sclerotia formed was recorded and the sclerotia were rated for size.

The response of the four cultivars to each of the four ergot isolates was identical to that obtained in our preliminary experiments and only the results of inoculations with an isolate from the "Manitou" cultivar are presented in Table 1. "Kenya Farmer" and "Carleton" were clearly more resistant than "Manitou" and "Stewart 63". Resistance is expressed by a decrease in the number of sclerotia formed, and a reduction in the amount of honeydew produced and in the size of the sclerotia. In the case of "Manitou" and "Stewart 63", the number of sclerotia produced per head is a fairly accurate index of the amount of infection. In the absence of sclerotia, normal kernels were found in the majority of the inoculated florets. In the case of "Kenya Farmer" and "Carleton", however, no kernels were found in florets where sclerotia were absent. Such florets contained small, discoloured and shrivelled ovaries or undeveloped kernels. It seems that the florets were infected and that further development of the fungus was inhibited. The nature of this reaction is still being investigated.

Table 1. RESPONSE OF WHEAT CULTIVARS INOCULATED WITH AN ISOLATE OF *Claviceps purpurea* FROM MANITOU WHEAT*

Cultivar	Percentage of florets with sclerotia§	Size of sclerotia‖	Amount of honeydew produced¶
Manitou†	70	2	3
Kenya Farmer†	26	1	1
Stewart 63‡	78	3	4
Carleton‡	42	1	2

* Inoculum concentration 10^4 conidia/ml.
† *Triticum aestivum* L. c.v.
‡ *Triticum durum* Desf. c.v.
§ Based on a total of five heads with ten inoculated florets/head.
‖ Visual rating of sclerotia size measured twenty-one days after inoculation.
(1) Sclerotia very small.
(2) Sclerotia approximately the size of normal kernel.
(3) Sclerotia larger than kernel, extending beyond the glumes.
¶ Visual rating of honeydew production measured ten days after inoculation.
(1) No visible honeydew.
(2) Honeydew confined within glumes.
(3) Honeydew exuding from infected florets in small drops.
(4) Honeydew exuding from infected florets in large drops and running down the head.

In the summer of 1969, "Kenya Farmer" was tested further at a disease nursery in conditions of natural infection and adjacent to a susceptible cultivar. In these conditions, a considerable number of sclerotia were observed in the susceptible cultivars. In "Kenya Farmer", however, only a few small sclerotia, of the size obtained in the greenhouse experiments, were recovered from a 20 foot row. Subsequently, an isolate was obtained from one of the "Kenya Farmer" sclerotia and used to inoculate this cultivar in the greenhouse. This isolate induced the same reactions as the previous isolates tested.

The resistance of "Kenya Farmer" and "Carleton" is expected to be adequate. Although infection can occur, honeydew production is very small and secondary infection should therefore be minimal. Furthermore, the fact that only low numbers of very small sclerotia are produced should reduce the amount of primary inoculum the next spring.

The resistance of "Kenya Farmer" was not influenced by the range of conditions in the greenhouse, and was maintained when plants were inoculated with some common biotypes of the fungus in our area. "Carleton" was not tested as extensively, but its resistance seems to be similar to that of "Kenya Farmer". Both cultivars are now being tested against a wider range of isolates, and studies on the inheritance of their resistance are in progress.

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Benlate-induced Instability of *Aspergillus* Diploids

BENOMYL is the proprietary name of the fungicide in which the active ingredient is benlate (methyl 1-(butyl carbamoyl)-2-benzimidazole carbamate). After extensive trials over the past few years it has been widely reported to be effective in controlling many phytopathogenic fungi and was released for commercial use in 1969. I wish to draw attention to experiments with *Aspergillus nidulans* which show that benlate radically alters the stability of diploid strains.

Heterozygous diploid strains of *A. nidulans* are easily selected[1] and occasionally form new diploid and haploid genotypes during vegetative growth[2]. These spontaneous segregants are formed after mitotic recombination and mitotic non-disjunction, and their occurrence is visually detectable in diploids heterozygous for genes controlling conidial colour. Segregants appear as distinctly coloured sectors in colonies grown from single uninucleate conidia. The diploid strain used in the experiments with benlate had green conidia, and because it was heterozygous for two genes

$$\left(\frac{W\ \ Y}{+\ \ y}\right)$$

affecting conidial colour both white and yellow segregants could be recognized. Conidia of this diploid genotype were spread on complete medium, and on complete medium containing benlate at the concentrations given in Table 1. Survival was estimated as the number of conidia which formed colonies, and colonies were scored as segregating if they contained either white or yellow sectors.

Table 1. EFFECT OF BENLATE ON SURVIVAL AND SEGREGATION OF A DIPLOID *Aspergillus* (bi-1 w(Y)/ad-9 methA-17 y)

Treatment	Survival		Colonies showing segregation	
	Number	Per cent	Number	Per cent
Control (no benlate)	87	100	4·7	5·4
0·25 p.p.m. benlate	75·3	86·6	31	41·2
0·5 p.p.m. benlate	42·7	49·1	30·6	71·6

Numbers are means of four replicates. Concentrations are given in parts per million. The gene symbols are those in regular use.

Besides inhibiting colony formation, benlate also markedly reduced the size of the colonies formed at the highest concentration. The sectors (segregants) were not only more numerous on the benlate containing media but also occupied a much larger area of the colony, indicating that in the presence of benlate the segregational events occurred earlier in the growth of the colony. Indeed, most of the spontaneous sectors consisted of only a few conidiophores. Further investigation showed that few of the spontaneous segregants were haploid (5/36), and that a larger proportion of segregants formed on 0·5 p.p.m. benlate were haploid (33/72). The corresponding proportions of haploid segregants from another diploid were respectively 3/26 and 48/51. Apparently benlate not only induced the formation of segregants but also significantly increased the proportion of haploid segregants. Benlate-induced sectoring was not detected in haploid strains, and no benlate-induced reversions of auxotrophic mutants were detected in the same strains. These results suggest that benlate may not cause gene mutations.

The effect of benlate is at least superficially similar to the induction of haploidization in *Aspergillus* diploids by *p*-fluorophenylalanine[3]. This is an antimetabolite of phenylalanine and its incorporation into cell proteins[4,5] is perhaps responsible for its production of aberrant spindles in dividing human cells[6]. Thus *p*-fluorophenylalanine is probably directly involved with chromosome segregation. Benlate may also operate directly on chromosome segregation, but its very different molecular structure indicates a different primary metabolic action. Mutagens that break chromosomes also cause instability of *Aspergillus* diploids[7,8]. Kafer[8] postulates that these have an indirect effect on stability. Chromosome breakage leads to the formation of nuclei hemizygous for the respective chromosome regions at subsequent nuclear divisions, and these nuclei are potentially unstable. Whatever the primary action of benlate, it evidently has genetic consequences in *Aspergillus*. Searches for possible cytological and genetical effects in other species are required in view of the likelihood that benlate will be widely used in agriculture. I have seen no reports of relevant experiments.

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Inhibition of Nematode Infestation of Wheat Seedlings by *Polygonum hydropiper*

SOME nematode diseases of crop plants are ameliorated by *Brassica*, *Tagetes* or *Asparagus* grown previously or concurrently in the same soil[1-5]. Toxic principles within their roots or exuded into the soil are thought to be responsible. Having observed that leaf and root extracts of *Polygonum hydropiper* L. have nematostatic properties against *Rhabditis* and other soil nematodes *in vitro*[6], I decided to study its effect on wheat disease caused by the wheat gall nematode, *Anguina tritici*.

Wheat seeds (variety "Red Coat") were surface sterilized, germinated at room temperature (28° C) and each sown in a pot (diameter 25 cm) of sterilized sandy soil supplemented with fertilizer. Sixteen seedlings of *P. hydropiper* at the eight or nine leaf stage (12–15 cm tall) were washed in distilled water and planted in eight of the pots. After 2 weeks wheat seedlings at the four leaf stage (6 cm tall) were transferred to all sixteen pots, those with *Polygonum* receiving two each and the rest four each. Half the pots containing *Polygonum* and wheat, and half those containing wheat alone, were inoculated with *A. tritici* (six galls/pot) and the remaining pots served as uninoculated controls. The pots were then soaked in water to soften the galls and release the nematode larvae which, although 10 yr old, contained active larvae. Within 4 weeks of inoculation heart leaves of some wheat seedlings were finely wrinkled and rolled and some stems were bent—symptoms of disease caused by *Anguina*[7]. After 7 weeks the intensity of disease was assessed. In pots without *Polygonum*, two wheat seedlings out of the four present were examined at random. Using the Baermann[8] technique many *Anguina* larvae were extracted from infested plants.

Table 1. EFFECT OF *P. hydropiper* ON THE INCIDENCE OF FOLIAGE SYMPTOMS OF WHEAT CAUSED BY *A. tritici*

Test	No. of wheat plants	Total leaves of wheat plant	Healthy leaves/plant	Diseased
Wheat only				
uninoculated	6*	97·8	All	None
inoculated	8	85·0 ± 12·0†	54·5 ± 7·9	36 per cent
Wheat plus *Polygonum*				
uninoculated	8	54·0	All	None
inoculated	8	52·6 ± 6·3	54·4 ± 7·8	4 per cent

* One pot lost. † Standard error.

Table 1 shows that most wheat leaves remained healthy when grown together with *P. hydropiper*. Nothing is known of the way in which *P. hydropiper* influences *A. tritici*, but possibly its root diffusates are toxic and prevent emerging larvae from moving and invading the host plants. Fewer leaves were produced by the wheat plants when they were grown together with *Polygonum*, probably because of competition for light, water and nutrients, but toxic root diffusates may also have been involved.

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Effect of Cobalt Applications to Pasture on Herbage Digestibility

THE importance and essential role of vitamin B_{12} and cobalt, its precursor, in ruminant nutrition and metabolism have been described[1-3]. Cobalt deficiency in sheep results in marked changes in the rumen microflora[4], and although some difference in the efficiency of fibre digestion may thus be expected to follow, there is little evidence to support this. Marston *et al.*[2] state that the rate and course of carbohydrate fermentation are unaffected by low cobalt levels in the rumen. Other workers have reported that cobalt supplements increased the digestibility of fibre[5,6], while Becker and Smith[7] found fibre digestibility to be reduced by cobalt supplementation, although the digestibility of ether extract and nitrogen-free extract increased.

This communication presents evidence of a substantial increase in digestibility after the application of small quantities of cobalt to pasture. Chemical analysis of Italian ryegrass (var. RVP) and perennial ryegrass (var. Reveille) showed both grasses to have a low cobalt content. Cobalt sulphate solution was therefore sprayed in early March on to half of each pasture (200 g Co per ha) (+Co) the other half being left untreated (−Co). Eight weeks after spraying, the grasses were harvested separately and artificially dried. The application of cobalt had no effect on herbage growth or on its botanical composition. Chemical analysis showed nitrogen (2·0–2·2 per cent) and fibre (26·5–28·6 per cent) contents to be similar for both varieties and to be unaffected by the cobalt application. Both varieties seemed to contain nutritionally adequate quantities of minerals other than cobalt, although Reveille showed a low content of calcium (0·27 per cent). Cobalt application significantly increased herbage cobalt content (Table 1) and, on the basis of published estimates[8], the untreated RVP was deficient, and the untreated Reveille marginally adequate, for lambs.

The artificially dried grasses were offered to four groups of six (three month old) lambs for nine weeks before digestibility was determined. No significant difference in the amount of feed consumed per day occurred between the groups offered the +Co and −Co herbages of either variety. For the first six weeks, lambs offered the +Co and −Co Reveille herbages gained weight at the same rate, but in the last three weeks the +Co group gained significantly more weight ($P = 0{\cdot}01$). There was no difference in the live weight gain of lambs offered the +Co and −Co RVP herbages.

After nine weeks the digestibility of each herbage was estimated, using three lambs from each of the four groups in the previous experiment. Faeces and urine were collected for eight days after a preliminary feeding period of ten days, and the lambs had therefore been offered the experimental feeds for over ten weeks before digestibility was determined. The digestibility of dry matter, organic matter and fibre was significantly higher ($P = 0{\cdot}01$) for the +Co than for the −Co Reveille herbages, but there was no difference in digestibility between the +Co and −Co RVP herbages (Table 1). There were no significant differences between treatments in intake of feed.

Table 1. COBALT CONTENT, DRY MATTER INTAKE AND DIGESTIBILITY OF THE EXPERIMENTAL FEEDS

			Mean values for three lambs		
Feeds	Co content (p.p.m.)	Intake (g $KgW^{0.73}$ per day)	Percentage of dry matter digested	Percentage of organic matter digested	Percentage of fibre digested
RVP +Co	0·25 †	69 *NS*	67 *NS*	70 *NS*	60 *NS*
−Co	0·06	59	67	69	62
S.E. of difference	0·01	4·3	0·50	0·68	1·96
Reveille +Co	0·27 †	58 *NS*	72 *	74 *	72 *
−Co	0·11	61	66	69	65
S.E. of difference	0·01	3·7	0·91	0·79	0·88

NS, Not significant.
* Significant difference at $P = 0{\cdot}01$.
† Significant difference at $P = 0{\cdot}001$.

It is clear that, in certain circumstances, the digestibility of a grass variety may be substantially influenced by changes in cobalt content. It seems that in the present experiments the increase in liveweight gain noted in lambs offered the +Co Reveille herbage may be attributed largely to its greater digestibility. The fact that the additional cobalt did not influence liveweight gain over the first six weeks of feeding suggests that the difference in digestibility arose after the sixth week and that the effect may be caused by change in the rumen flora. A surprising feature of the results is that further cobalt did not affect the digestibility of the RVP ryegrass, although it contained less cobalt than the Reveille. This may indicate a difference in the availability of cobalt in the two varieties, and requires further investigation.

These experiments are being continued, but there are several implications already. In certain circumstances, the provision of extra cobalt seems to stimulate rumen activity and to increase fibre digestion. In herbage evaluation using *in vitro* digestion techniques, changes in the digestibility of varieties as a result of mineral deficiencies may not necessarily be found, for the inoculum used may be rich in cobalt. Finally, improved animal production may be possible by the application of trace amounts of cobalt to certain herbage varieties.

I thank Professor P. T. Thomas for his interest in this work and Mr Roy Hughes and his colleagues in the Agronomy Department for their collaboration.

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Book Reviews

GROWTH CYCLES

Is the Business Cycle Obsolete?

Edited by M. Bronfenbrenner. Pp. xii+567. (Wiley (Interscience): London and New York, March 1970.) 120*s*.

Economists and economic historians have long been fascinated by the way in which economic activity tends to fluctuate over time, and many theories have been put forward to explain cyclical behaviour. The papers in this volume (an outcrop of a conference held in London in April 1967) are primarily concerned, however, with the question of whether the business cycle is now defunct. The selection includes a number of case studies of individual countries, both capitalist and socialist, three econometric studies and two general articles on business cycle policies and the transmission of fluctuations from developed to underdeveloped countries. It is an impressive collection of papers written by distinguished scholars.

The general consensus of opinion seems to be that business cycles are still with us but that their characteristics are somewhat different from those before the war. Broadly speaking, cycles are shorter and less intense than they were previously, and in many cases high floors in the downswing have resulted in what might be termed "growth cycles" in which the recessions are characterized by decelerations in rates of growth rather than by absolute contractions in economic activity. Even these modified cycles may be on the way to extinction given the progress in macroeconomic and econometric model building and forecasting.

It is impossible to summarize briefly the great deal of information contained in this volume and, in any case, there is a long and very competent survey of the whole proceedings by the editor at the end of the volume. Unfortunately, the discussion of the debate is on a chapter by chapter basis, and only a very sketchy attempt is made to draw the common threads together in the preface. This is a serious shortcoming, because the contributions do vary a great deal in quality, scope, methodology, and in their conclusions. The most useful paper is Bert Hickman's elegant survey of econometric models, while the least informative are those by Nove on the Soviet Union and by Dow on France, Germany and Italy. Only a few authors put the post-war cycle in its long-term historical context, and in this respect Professor Matthews's paper on Britain is one of the best. The methods used to measure the amplitude of cycles and date their turning points vary considerably, and it is a pity that some effort was not made to achieve a greater degree of uniformity in this matter. Rather surprisingly, there is no specific paper dealing with the transmission of cycles among developed countries—a rather serious omission in view of the fact that it would have helped to clarify the issue of the relative mildness of the post-war cycle. Fortunately, an excellent chapter by Kurt Rothschild on Austria and Switzerland, a title which somewhat belies the wider context in which it is set, helps to repair this gap to some extent.

Perhaps the chief defect of the volume stems from the fact that the authors have been too concerned with presenting the facts and relating these to the conference title, to the ultimate neglect of analysing the reasons for the changes in cyclical characteristics in the post-war period. Nevertheless, the work as a whole provides a useful insight into the type of business cycle research now in progress.

DEREK H. ALDCROFT

URBAN DEVELOPMENT

Developing Patterns of Urbanization

Edited by Peter Cowan. (Centre for Environmental Studies.) Pp. vi+216. (Oliver and Boyd: Edinburgh, February 1970.) 40*s*.

Economic, social and physical development is proceeding so fast now that the importance of forecasting the future is being understood to an increasing extent. This volume contains an attempt by a group of specialists in fields related to urbanization to explore to what extent guidance can be given in this subject by a forecast of possible future developments. This has resulted in a collection of essays which, according to one contributor, "amounts to a lightly disciplined exercise in intuitive thinking; hopefully the next phase will introduce greater rigour and sharper purpose" (D. Bayliss). This is a somewhat modest statement, underestimating perhaps the significance of fascinating and thought provoking contributions. To forecast the future is indeed a difficult task, and it is not surprising that a group of distinguished writers does not necessarily reach a consensus of opinion; it may be judged in fact to be one of the merits of the essays that the reader is presented with differing alternatives, and so encouraged, if he is interested in and concerned with urban problems, to evolve his own interpretations.

It seems very likely that the broad pattern of urbanization, which has not been changed much in the geographic sense during the past 50 or even 500 years, will look not so very different in AD 2000, existing investment in the built environment being a compelling constraint (E. Jones and P. A. Stone). Besides, people are not prepared to spend as much on housing as they do, for instance, on motoring, and may well favour a greater output of restored and reconditioned dwellings which can be produced quicker and cheaper and will therefore command lower rents. In any case, there are doubts as to whether the space economy practised since the Second World War has produced housing which will be sufficiently adaptable for future needs; the oldest section of the housing stock will probably not be the first social slums of the near future (C. Price). But the expected population growth cannot be accommodated entirely in existing cities, nor, with some exceptions, in self contained new cities: it will have to be retained in the suburban ring of existing metropolitan areas (P. Hall). Such a pattern will meet the clear demand for more space both near the home and in a not too distant countryside; it will permit central decongestion and follow well established trends of decentralization along fast transportation radials on a regional scale and perhaps reconcentration at the local suburban scale. Such a trend may be accelerated by developing traffic technology, increasing speeds and improved telecommunications (P. Cowan).

Considerable changes in life styles and activities can take place, however, without a change in the physical pattern of our urban areas. There seems to be a growing popularity of family life related to a shift towards the home and marriage partnership. More people, including skilled and unskilled manual workers, buy their own homes. People get married much earlier, which means that their grown up children will leave them on their own for very much longer periods than was the case in earlier generations (P. Wilmot). But, whereas at the child rearing age suburban homes may be preferred, at the subsequent stage a central home with ready access to work for the wife may be preferred. Many families in central areas would like a second home in the country; could it be that the home in the central area could become the second home with a family base in the suburb or in the country? (A. Price). Will the coloured population be permitted an adequate place in the evolving pattern? (N. Deakin). Provision for recreation will be an issue of increasing importance (B. Rodgers). Leisure activities are more likely to be affected by developing life styles rather than by income or working hours; as is well known, many work overtime or take second jobs. But will it be necessary to invent "pseudo work" as a leisure activity? (D. Bayliss). It is a strange belief in our society that western man must always be active; we pay lip service to the civilizations of the East, but can we not learn from them: contemplation?

Finally, there are some important comments on the approach to "planning" itself. Goals should be precisely articulated and should be subject to public debate (A. G. Wilson). The relation between the planners and the planned may well become one of the most crucial and decisive questions during the next few decades (P. Cowan). All the contributors seem to have accepted as unalterable the considerable population growth forecast for the end of the century; but one would like to think that a limitation of population growth in the interest of the community as a whole could also become an important political issue.

It is the aim of a reviewer of a deserving work to stimulate the interest of the readers; I hope I have succeeded in doing so.

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WHAT IS CYBERNETICS?

Survey of Cybernetics

(A Tribute to Dr Norbert Wiener.) Edited by J. Rose. Pp. xi + 391. (Iliffe: London, January 1970.) 105*s*.

ONE of the contributors to this book writes aptly, "We have covered a great deal of ground and have done it rather unevenly". Although to the editor of the book, Wiener's contribution to cybernetics stands supreme, it is Wiener's definition which makes another contributor ask, "What is cybernetics? Or rather, what is it not?"

Boulanger claims that Ashby's homeostat foreshadows the future auto-pilot. Surely, any such random-trial, non-learning machine would crash most planes? But to overlook completely the features of randomness and probability in robots, as do some of the contributors, would seem to be just as wrong as making them paramount.

Several contributors concentrate on the listing of definitions. Perhaps this is useful, but should not practical results come first? As Walter states, "This peculiar gap between theory and practice is a feature of cybernetics". One welcomes Walter's criticism of Wiener, because it is only with the spread of such a critical attitude that further progress can be expected.

Clark's review of medical cybernetics is most useful, but Rosie's chapter on information theory seems rather out of place. At last, after fifteen years of secrecy, Pask publishes some details of his early work on learning matrices, but by now this is of historical importance only.

Apter suggests that his particular segment of the field should become more central to cybernetics, in spite of its deterministic, non-probabilistic nature. Leondes and Mendel review artificial intelligence control, but admit that the widely used single linear threshold element is "off-line". Should not then the cybernetic validity of all such work be questioned, because clearly any animal learning system must always be "on-line"?

The editor of the book seeks to eliminate the fragmentation of scientific thought and activity. But is it not this very fragmentation which has, in the past, produced scientific progress? Do we really want to impose a somewhat totalitarian structure on technology, by such steps as "The reduction of the numbers of technological societies"? What is really needed to unify the technologies, the editor writes, is a common cybernetic basis.

Surely, it is important not to ignore the fact that if we allow any attempt by any national, or worse still by any new international, body to impose any such unification, then scientific progress is likely to cease, for progress implies freedom and the absence of regimentation.

This book has helped to confirm the suspicion that between those who actually pursue active research work in practical cybernetics, and those who merely write and talk about it, there is a widening barrier. For we are told in the book that it is cybernetics which "would forge various disciplines into a unit that is part of the human search for truth and for understanding of man's place in society".

One would suggest humbly that there is no need to apply the name of cybernetics to this, because a more suitable name is in existence. The process is more usually simply called "science".

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CELL TO CELL MESSAGES

Mediators of Cellular Immunity

Proceedings of an International Conference held at Brook Lodge, Augusta, Michigan, April 28–30, 1969. Edited by H. Sherwood Lawrence and Maurice Landy. (Perspectives in Immunology: a Series of Publications based on Symposia.) Pp. xv + 469. (Academic Press: New York and London, December 1969.) 89*s*.

MUCH the most profitable kind of meeting for furthering an area of experimentation is one restricted to about thirty expert participants who have no closely set programme. A volume compiled from prewritten pieces is then quite inappropriate and is a custom that increasingly discourages attendance at symposia. The present volume is the best answer found so far for conveying the resulting wisdom to those not fortunate enough to have been invited, and whose very numbers in attendance would have inhibited the searching discussions and criticisms that are of such value.

This book is essential reading for all immunologists, mentally indigestible though it is. The many criticisms as a readable book must be excused by what it achieves, even its typographical errors by its speed of publication. The confused state of the subject is the reason for its choice and it can be likened to a detective novel where clues are overabundant, some false, but at the end the criminals are not brought to trial. Thus there is no summary; you read it all or you do not read it.

It is astonishing that a free ranging discussion can lead to such a good overall sequence; in this respect it is an improvement on the first volume of the series and reflects admirably upon chairmen and speakers alike. The whole content is alive and complex; Dr X ends a piece with ". . . I don't know if that answers your question". Dr Y replies "I am not sure either. . .". References are provided sparsely in some parts of the text, other parts could profitably have included as many.

I submit that for conveying the gist of the content this is a particularly difficult kind of volume to deal with. There are six sessions reported verbatim, each excellently introduced by a speaker who seeds the congregation with chips from the particular axe he grinds. In the last 18 pages chairmen attempt to summarize their sessions, but this is disappointing; it is not summarizable material. Particularly interesting discussions cover the following important areas. Session one, events preceding the appearance of blast cells when lymphocytes are stimulated *in vitro*; the commitment of 1 to 2 per cent of a cell population in cell mediated immunity is compared with a maximum of 0·01 per cent of cells destined to secrete antibody. Only the latter figure is consistent with clonal selection, some form of which most immunologists would like to settle for. Session two includes the hypothetical nonsecretable IgX that, if it exists, would bring recognition for cellular immunity in line with that for humoral responses; cell co-operation in thymus dependent populations and the role of non-thymus dependent lymphocytes and of macrophages are discussed. Session three has all the data on transfer factor that is below 10,000 molecular weight but seems to transfer specific cellular responsiveness between individuals. Is it some kind of immunogen or do we not yet have a pigeonhole to file a molecule like that ? It can now be compared with the "factors" discussed in sessions four and five that are released from stimulated cells *in vitro*. There may be seven (migration inhibitory, lymphotoxic, skin-reactive, chemotactic, mitogenic, plus interferon and antibody), but we may hope that apparent differences may reflect the different test systems used and there may be fewer. Finally, session six considers the difficulty of having a foolproof surveillance system that would distinguish between an unwanted tumour cell and a self + X entity leading to self damage arising from delayed hypersensitivity.

The overall result is a sorting out of what evidence is conflicting, what are crucial points to establish, what alternative interpretations might be put on results that conflict with the most plausible hypotheses, and what weak points can be discerned in the defences where best to apply renewed experimental pressure.

D. A. L. DAVIES

ACTION OF ANALOGUES

Analogues of Nucleic Acid Components

Mechanisms of Action. By P. Roy-Burman. (Recent Results in Cancer Research, Vol. 25.) Pp. xi+111. (Springer-Verlag: Berlin and New York, 1970.) 28 DM; $7.70.

DURING the past twenty years large numbers of analogues of purine and pyrimidine derivatives have been synthesized as compounds of potential therapeutic value and there has been a large volume of research effort devoted to elucidating the biological activity, metabolism and molecular modes of action of these substances. The results of these researches are scattered widely throughout the literature and this monograph is of value in that a large amount of information has been assembled and condensed by the author into a single slim volume.

The book includes brief, but well illustrated, summaries of the biosynthesis and degradation of purine and pyrimidine nucleotides. A representative selection of the more important purine and pyrimidine analogues is considered, and in each case the evidence relating to the metabolism of the compound, its effect upon enzyme systems and, where appropriate, the consequences of its incorporation into nucleic acids are reviewed briefly.

The author has managed to condense a great deal of information into a remarkably small amount of space. While the presentation is somewhat concentrated, it is fully documented and well supported with diagrams illustrating on the one hand the metabolism of the analogues and on the other the reactions with which they, or their metabolic derivatives, interfere. This compilation will be a useful source of information to many scientists in the field of cancer therapy and to those with more general interests in the area of nucleotide and nucleic acid biosynthesis and metabolism.

While the circulation of a highly specialized publication such as this will inevitably be small, it is unfortunate that it should have been so highly priced. At a more modest figure the book might have been attractive to many individual purchasers and would certainly have been much better value for money.

R. M. S. SMELLIE

ESSAYS ON BOTANY

Cellular Differentiation in Plants and other Essays

By C. W. Wardlaw. Pp. 160. (Manchester University Press: Manchester; Barnes and Noble: New York, March 1970.) 40*s*.

FOR fifty years Professor Wardlaw has been a keen student of plant morphology and morphogenesis. Readers of his previous books, such as *Phylogeny and Morphogenesis*, *Embryogenesis in Plants*, and more recently (1965) *Organization and Evolution in Plants*, will be familiar with his interests, as well as his predilection for the philosophical aspects of biologic organization. This new book is a collection of five essays, or "emeritus excogitations", in Wardlaw's phraseology, written in the past few years on subjects dear to his heart. In one of the essays Professor Wardlaw tells of the reception his *Organization and Evolution in Plants* received at the hands of the critics: "there was one sapient chap who indicated that I had explained very little and that all I had given my readers was words". I imagine that that same reviewer would have said something similar about this book. But yet, there are still challenging questions in plant morphogenesis that lie along "the dangerous, jungly pathways which I, in my still youthlike enthusiasm, feel impelled to tread", and we, the readers, should follow —at least a little way.

This book is not for the impatient reader after hard, clear facts; nor is it a propaedeutic book easily accessible for the interested beginner, as the dust-cover rubric states; rather, it is for one familiar, and preferably in empathy, with Wardlaw's earlier writings. The longest essay of the book, entitled "Aspects of Cellular Differentiation", is in two parts. The first gives us a historical perspective of the subject, reminding us that the current view relating a cell and its milieu to the developmental pathway it will take, was anticipated by the botanists of old (Grew, Vöchting, Driesch). The second part considers modern aspects of differentiation. Here Wardlaw relies largely on Bonner's book *The Molecular Biology of Development* for his attempt to see how far molecular biology can be profitably used to interpret morphogenesis and epigenesis.

If cell differentiation is "one of the most important, beguiling, provoking and difficult themes in botany" then what of epigenesis, the topic of the fourth essay ? It is "just plain enigmatic". Enigmas arise out of ignorance: we need to think more deeply, more clearly, or in new directions if they are to be dispelled. But in which direction ? For instance, if we seek the aid of the molecular biologist should he be concerned with the chemistry of atoms and electrons or with macro and megamolecules ? Wardlaw recognizes the challenge but does not give any guidance or new ideas. Some may think that he deliberately invokes obscurity by introducing into his thesis the intangible Aristotelian concept of entelechy, and by speaking of "the deep inwardness of cells during their growth and development . . . that makes our understanding of them more and more remote", as well as by injecting a touch of unorthodoxy by mentioning that cells may transcend their genetic potentialities in creating neoformations.

Perhaps the most successful essay is the third, "Reconciliations among Apex Lovers". Wardlaw attempts to resolve rival points of view about the structure and function of shoot apical meristems that still prevail. His criteria for reconciliation are nothing more than the call for scientific objectivity and it seems an unfortunate reflection on those working in this field that their conflict of views attracts an essay of this kind.

Professor Wardlaw's style of writing is unique. It is difficult to decide whether it is unforced literariness, or whether he writes with Robin Goodfellow at his elbow. If the former, the product is eccentric and the effect is irritating; if the latter, then the following passage from the first essay "On Writing Botanical Essays" is a gem of self-parody: "When I was a mere botanical neophyte, in the early spring sunshine of promise, in fact at the ripe old age of twenty, every new volume that my revered Professor commended to me and, indeed, was often kind enough to put into my hands, seemed to me to exhale a rare, exotic perfume, a distilled fragrance, nay ! the very elixir of true learning". If the botanical neophytes of today, as well as those of maturer years, can grope through the exotic perfumes exhaled by this book they will find something. But that something they will probably have thought of for themselves or have read elsewhere, at less length, in books of more substance.

PETER BARLOW

TRIBUTE TO A BOTANIST

J. Sen Memorial Volume

Edited by H. Santapau, A. K. Ghosh, S. K. Roy, S. Chanda and S. K. Chaudhuri. Pp. xxi + 498. (J. Sen Memorial Committee and Botanical Society of Bengal: Calcutta, 1969.) Rs. 40; $10.

INDIA has had the misfortune to lose several of its distinguished botanists in the prime of life. Birbal Sahni, FRS, died before he was 58; P. Maheshwari, FRS, soon after 60; and Jitendrakumar Sen, deputy director of the Botanical Survey of India, died in December 1966 at the cruelly premature age of 45.

This book, published to perpetuate Sen's memory, begins with a tribute from John Walton, Emeritus Professor of botany in the University of Glasgow, followed by a short biography provided by Dr Sunirmal Chanda, Bose Research Institute, Calcutta. Dr Sen's publications are then listed, sixty-eight in twenty-three years, testifying to his industry. His contributions lay chiefly in the fields of palynology, palaeobotany and wood histology, later turning to horticulture. Appropriately, the fifty-eight articles making up the bulk of the volume are grouped into four sections: palaeopalynology, palynology (Recent), palaeobotany and taxonomy and other topics.

Sixteen papers on palaeopalynology, chiefly from Indian authors, describe spores or pollen from the Cambrian to the Quaternary. A. K. Ghosh and A. Bose record "vascular microfossils" from North American Cambrian rock samples supplied by Professor B. F. Howell of Princeton University. The authors provide no illustrations. Without these it is difficult to evaluate their claim that "it is obvious that the vascular plants had an origin long before the Silurian period". G. O. W. Kremp and J. G. Methvin discuss the increasing problems of data retrieval in palynological research and suggest that the application of computer techniques can save the situation and promote fruitful research on a world scale.

The section on Recent palynology has seven contributions: one by G. Erdtman and A. Dunbar on the fine structure of *Macrozamia* pollen, the rest by Indian contributors on the pollen of Scrophulariaceae, Casuarinaceae, Gardeniaceae and Sterculiaceae, and on Indian fern spores.

Of the twenty-one palaeobotanical papers, eighteen are by Indian authors and most of these deal with floras or individual taxa ranging from the Lower Gondwanas of India to the Tertiary. Of particular interest here is Manju Banerjee's paper on *Senotheca*, a new fructification, associated with leaves of *Glossopteris taeniopteroides* Feistmantel, from which cuticularized seeds were obtained by maceration. In the same section Emeritus Professor Tom M. Harris discusses the problems of naming Jurassic conifers and Dr Edna Plumstead reflects on the earliest plant life.

The last section has fourteen papers, all by Indian authors. Subjects covered include cytology, chemotaxonomy and pharmacognosy applied to taxonomy, systematics of various Indian plants, floristics, conifer wood structure, and lastly an account of the Dehra Dun Herbarium by Professor Emeritus M. B. Raizada.

The book is set in easily readable type and cleanly printed on rather poor quality greyish paper. There are commendably few printing errors. Text-figures are excellent, but some of the plates are poor, notably those between pages 152–153 and 208–209, where alternating dark and light vertical bands make it difficult to understand spore ornamentation. Apart from these small technical blemishes, the book is a praiseworthy production and a fitting tribute to one who did much in a short working life to advance the cause of Indian botany.

WILLIAM S. LACEY

SPIDERS, SCORPIONS AND MITES

The Arachnids

An Introduction. By Keith R. Snow. Pp. 84. (Routledge and Kegan Paul: London, April 1970.) 25*s* boards; 14*s* paper.

THE word Arachnida has recently appeared in the syllabuses of several examining boards, and this book is a timely response to the need for more information than is contained in the ordinary textbooks.

An introductory chapter describes the general structure, outlines the physiology of the class, and ends with short diagnoses of the six orders chosen for fuller treatment. First place is then given to the Acari, which are scarcely typical arachnids, but which occupy twenty pages, or a quarter of the whole. Perhaps the economic importance of ticks and mites is held to justify this proportion. Spiders and scorpions together account for another quarter.

Dr Snow has kept prominently in mind the needs of examination candidates, and this may be taken to explain the meagre space given to the other orders. A better balance might have been obtained by shortening the glossary: readers for whom this book is meant should not need definitions of such words as lateral, marine and prey, and many others. But compression was essential, and is regrettable only when particular statements appear to be generalizations. Obviously, exceptions cannot often be mentioned, but the implication that all spiders' webs are orb-webs should have been avoided. There are a few mistakes: for example, Fig. 18 gives a spider four lung-books, and the remark that harvestmen mate two or three times a season is an understatement of their remarkable promiscuity.

Some readers will be surprised and others pleased at the inclusion of *Limulus*, an animal which, though not a true arachnid, can undoubtedly teach us much that has not yet been learned from its study. Many, too, will appreciate the advice on fieldwork and laboratory methods.

A word of praise is due to the illustrations, admirable line drawings that show precisely all details of structure. The book, in fact, is successful as just what it set out to be—not a monograph, but a helpful introduction to a group that is beginning to receive that appreciation it has always deserved.

THEODORE SAVORY

ADRENAL PATHOLOGY

Functional Pathology of the Human Adrenal Gland

By Thomas Symington. Pp. xvi + 551. (Livingstone: Edinburgh and London, 1969.) 200*s*.

THIS book contains a wealth of information about the adrenal gland as is only to be expected from its chief author, Professor T. Symington. He states that he has tried to avoid the temptation to produce just another book with a descriptive catalogue of the pathology of the adrenal gland but to produce a more dynamic approach to adrenal disease based on the knowledge of normal structure and function. In my opinion he has certainly succeeded.

The book commences with a very large section on the adrenal cortex, describing features of the foetal, neonatal, infant and adult stages of this gland. The biosynthesis and metabolism of steroids are discussed, and the physiology and pathology of the cortex both in health and disease are more than adequately covered. Technical and chemical methods used for the investigations of disorders of the gland are also given. I am pleased to see that the author aptly points out that investigations of the biosynthetic ability of adrenal tissue by radiochemical incubation studies *in vitro* need not reflect what is happening *in vivo*. More *in vivo* studies should certainly be made where possible.

The next section of the book deals with the adrenal medulla by A. M. Neville and again the same pattern of describing the foetal and adult gland followed by the biochemistry, physiology and pathology is followed as for the cortex section. An excellent chapter on the detection and measurement of catecholamines in tissues and body fluids ends this section.

A section on the chemoreceptor system follows. At first this might seem to be out of context in a book about the adrenal gland, but the demonstration of catecholamines in the normal carotid body and in some tumours arising from it together with the possible neuroectodermal contribution to its development serves, I suppose, to emphasize the close relationship between the chemoreceptor and chromaffin systems.

A remarkable section devoted to an atlas of human adrenal cortex ultrastructure by A. MacKay now follows. This atlas, which is divided into three parts, gives well detailed electron micrographs of the three zones of the normal human cortex, then micrographs of the pathological human adrenal, and finally details of the processing techniques are given. Altogether more than a hundred micrographs are presented, which makes the book rather unique. Much of our present knowledge of steroid biosynthesis has been acquired from tissue slices or homogenates of normal and abnormal adrenals, and the electron microscope shows that all such preparations have a much greater structural heterogeneity than had been suspected by light microscopy. While the cells involved in normal and abnormal steroid biosynthesis have, however, been better characterized, many problems on the correlation of structure and function of the adrenal cell still exist. It is nice to see that the major drawbacks of interpreting the electron micrographical data are pointed out.

The book finishes with a short section on the metabolic and hormonal response of the body to injury.

This book, though intended primarily for pathologists, will interest biochemists and endocrinologists. It seems rather expensive, but with its 550 pages with its excellent atlas of human cortex ultrastructure I believe it to be well worth the money. D. EXLEY

DECOMPRESSION SICKNESS

Subatmospheric Decompression Sickness in Man

By D. I. Fryer. (AGARDograph No. 125.) Pp. 343. (Technivision: Slough, 1969.) 145*s*.

IT is surprising to hear that in spite of all the advances in aircraft technology resulting in the provision of reliable pressure cabins and operational pressure suits, the hazards to man of subatmospheric decompression sickness have not decreased.

This book admirably illustrates the value of taking a critical overall look at a subject. It gets the problems into perspective and in this case reveals how fragmentary our knowledge is.

In an effort to determine precisely where we are in our understanding of subatmospheric decompression sickness the author, who is a serving medical officer in the Royal Air Force, has assessed with remarkable perception a multitude of papers which have been written on this subject. In his evaluations he has made extensive use of his own practical experience and experimental observations. It is clear that we undoubtedly possess a lot of knowledge about decompression sickness but very little understanding, and the author rightly comes to the disappointing conclusion that "there can be few subjects as frustrating as that of subatmospheric decompression sickness, for its manifestations are so protean, its mechanisms so obscure and its prevention so difficult in practice".

For those who may wish to take up the challenge and to give serious consideration to this intriguing problem there is a wealth of informed opinion in this book and an excellent bibliography of more than 450 references.

D. N. WALDER

JOINT ACTION

Lubrication and Wear in Joints

Edited by Verna Wright. (Proceedings of a Symposium organized by the Biological Engineering Society and held at the General Infirmary, Leeds, on April 17, 1969.) Pp. 152. (Sector: London, March 1970. Distributed in the USA and Canada and South America by J. B. Lippincott.) 53*s*.

THIS collection of papers delivered at a symposium held about a year ago represents the thinking at that time of research groups, chiefly in the United Kingdom, on the subject of lubrication of diarthrodial joints. The newest theory, that of "trapped pool" lubrication, is given the greatest coverage. Observations on the structure of articular cartilage as interpreted by electron scanning are reported in some detail and excellent illustrations are provided in support. Those who support other theories of lubrication also had their say, both positively and, through the discussion, in challenging the validity of the data on which the trapped pool theory is based. A contribution on artificial lubricants, the last chapter, is spoiled by the omission of the table of criteria which a lubricant should fulfil before it could be used in an animal joint. Nor is there any evidence to support the premise that artificial lubricants could halt the disintegration of articular cartilage once fibrillation is established in weight-bearing areas.

The chapter on joint disease and the causation of osteoarthritis is disappointing in that it presents a standard list of causes which has been in use for many years and does not indicate that any new thinking about the subject is being indulged in by any of the groups represented at the meeting. There were no contributions dealing with the subject of wear of articular cartilage, but the impression is left that "wearing out" of cartilage as a purely mechanical process can be an important cause of osteoarthritis. It is evident from these papers that bioengineering research groups should have one member who is a biologist. Although the discussions applied some correctives there is no substitute for a built-in stabilizer.

Chapters are also devoted to joint prostheses and joint simulators—the problem of wear is a very real one when dealing with joint prostheses.

Elegant analyses of the forces acting on the hip and knee are presented by Paul. This work is the best dynamics study of joint forces so far available and must be required reading for everybody interested in joint function. We must also learn the lesson that the magnitude of force changes rapidly when the normal person is ambulant and is not accurately simulated by many of the compression studies carried out on cartilage to date.

As a deliberate policy an index has been dispensed with and bold marginal sub-headings have been used to indicate important topics. This has produced an attractive arrangement for reading, but the reason for choosing some of the subheadings is not clear, and an index would have improved the presentation. To compensate, there is an extensive list of pertinent references in an appendix. Photographs and diagrams are excellent throughout.

As is true for most symposia records, this one will be of most value for those wishing to become acquainted quickly with the principal lines of investigation. Clinicians and biologists will probably derive most benefit, while engineers may conclude that there is still a lot to be learnt about the physiology of diarthrodial joints.

C. G. Woods

NEW NUCLEAR TEXT

Nuclear Electronics

By E. Kowalski. Pp. x402. (Springer-Verlag: Berlin New York, 1970) 96 DM; $26.40.

I used to start my courses on electronics by ritually smashing a valve with a hammer. I don't do it now because there was always a slight danger in the procedure and the message about transistors has reached all but the most reactionary quarters. If, and it must be said that the style, presentation and general quality of production are a formidable inhibitory influence, one were to extend the principle to the work by Dr. Kowalski, there would be little to do for the book is up-to-date and illustrates most of the techniques with diagrams showing integrated circuits or transistors.

The author starts by giving the theoretical background to radiation detectors but in this, as in the work as a whole, he fully illustrates the argument with circuit diagrams. In the introduction he had made the point that it is never too soon to get away from the vagaries of analogue circuits into the sweet certainty of digital representation and in the middle section he makes much of the tools, both technical and mathematical by which the transformation may be accomplished. The last section of the work contains a comprehensive review of digital techniques complete with a note on Boolean algebra.

As small digital computers are changing the approach to the problem of processing digital information at the same frenic rate that transisters have changed the methods and scope of instrumentation, it would be churlish to complain that the section on them is too short, particularly in view of the quality of the background information on digital circuits in general. Nevertheless it is possible to see that this section should, in future editions, be enlarged.

It is stated in the preface that one of the aims of the text is that it should be useful as a reference work in years to come and the extensive references must alone make this a strong possibility. The list includes more than 900 entries most of which have been published within the last three or four years. Altogether the book is to be recommended as an important and timely addition to the literature of the subject.

Lindsay Molyneux

This review has been set by the combination of a 'Micro 16' computer (Digico Ltd) directly interfaced with the photo-unit of a 'Linotron 505' typesetting machine. The computer, as well as deciding the line endings and adjusting the spaces for justification, also simulated the functions of the logic unit of the 505. Because the first proof (set in 39 seconds) contained an error, a second proof was made using the ADDATAPE correction system; this took 44 seconds. The programs and interface were developed by Dr Molyneux in the School of Physics, University of Newcastle upon Tyne, during a Ministry of Technology Computer Typesetting Research Contract.

ORGANIC CHEMISTRY REVIEWS

Topics in Carbocyclic Chemistry

Vol. 1. Edited by D. Lloyd. Pp. xi+373. (Logos Press, in association with Elek Books: London, 1969.) 125*s*.

In recent years an increasing number of publications devoted to reviewing specific areas of organic chemistry has appeared. These are, on the whole, welcomed by organic chemists because they relieve to some extent the ever-growing burden of the original literature. This book is the first of a series which is to provide reviews in the field of carbocyclic chemistry. The series is entitled "Topics" rather than "Advances" because the articles are intended to "present the subject with sufficient background to make them acceptable to all readers". In my opinion this distinction is somewhat pedantic because most comprehensive reviews, even of the recent literature, would more or less satisfy this demand.

The necessity for a publication of this type is also debatable because its usefulness to a particular chemist will be severely limited by the enormous scope of the field of carbocyclic chemistry. Of the articles in the current volume, that on the benzidine rearrangement, for example, could easily have found a place in *Advances in Physical Organic Chemistry*, and that on bicyclo[3.3.1]nonanes would have been suitable for *Advances in Alicyclic Chemistry*.

Aside from this carping, the book, which is excellently produced, will be a valuable addition to any chemical library. The standard of the individual articles is high and they mostly cover the literature up to 1968 with more recent work in addenda. D. V. Banthorpe in "The Benzidine Rearrangement" provides a comprehensive and critical account of this problem which has now beset chemists for more than a hundred years. D. H. G. Crout, in "The Biosynthesis of Carbocyclic Compounds", has performed the difficult task of compressing a vast amount of material into an intelligible account. Of necessity there are various incursions into the domain of heterocyclic chemistry. G. L. Buchanan, in "Bicyclo[3.3.1]-nonanes and Related Compounds", entertains the reader to a lucid account of these interesting systems, and D. M. G. Lloyd provides a short article on "Feist's Acid".

H. P. Figeys, in an article entitled "Electronic Structure and Spectral Properties of Annulenes and Related Compounds", presents a critique of the theoretical chemistry of the annulenes which is both readable and intelligible to the experimental organic chemist. Particular attention is devoted to antiaromaticity as is merited by recent interest in this area. Breslow's speculations on the fusion of two 4n systems are dismissed as "two wrongs do not make a right". Much discussion is devoted to the red herring of benzo-annelated systems whereas the annulenyl and annulenium ions are unfortunately entirely omitted.

M. V. Sargent

REFRACTORY METALS

Properties of Refractory Metals

By Walter D. Wilkinson. Pp. x+355. (Gordon and Breach: New York and London, November 1969.) $33; 275*s*.

The text of this book was completed in May 1967 (but not published until November 1969) under the direction of the American Society for Metals on behalf of the United States Atomic Energy Commission. It is consequently a book on refractory metals for use in nuclear reactors and covers vanadium, chromium, niobium, molybdenum, tantalum and tungsten. A somewhat pedantic choice of a lower melting limit of 1,855° C for deciding the metals to be discussed results in the exclusion of zirconium (m.p. 1,852° C) and the inclusion of chromium (m.p. 1,875° C). This seems to be straining to rationalize a choice that could otherwise have been made straightforwardly and, by omitting zirconium, it results in the book being less useful to technologists in the nuclear power field than it need have been. Although this is a minor point, it does seem to reflect the author's approach to his task, which is an uneasy attempt to combine the academic (with its aridity for the practical man) with the practical (with its more than occasional fatuity for the high flown academic).

The author has his academic fling in the first five chapters in which there has been some hard work to squeeze generalizations from the plethora of experimental data on these metals. The first chapter is called "Atoms, Electrons and Fabricative Properties", and, as might be expected, is an odd mixture. After pages which deal *inter alia* with *d*-electrons, N(E)–E curves, bandwidths and Bragg disordering energy, we come down to earth with "proper thermal conductivity is essential for any material used as cladding or jacketing* for nuclear fuels". While wondering what "proper" thermal conductivity is, the reader's eye seeks the asterisked footnote, to be told "cladding" is a coating or layer of metal metallurgically bonded to a substratum. " 'Jacketing' (often called 'cladding' in the nuclear energy field) is sheathing that is not metallurgically bonded" (p. 25). Such a combination of the slipshod with the trivial reduces faith in much of the remainder which, being a recital of experimental facts, must be taken at its face value.

The next four chapters are on "Corrosion, Compatibility and Protective Coatings", "Interstitials and their Effect on Properties", "Ductility and Brittleness" and "Mechanical and Deformation Characteristics".

Two chapters are then devoted respectively to the tantalum and tungsten group metals. The final chapter is on heat treatment. Each of these contains more information of the sort presented in the earlier chapters but with less of the academic fluff.

To me, the book appears as an inappropriately organized and indigestible presentation of detailed experimental data. It is an expensive buy which could have benefited from a tough and critical editor. J. G. BALL

REACTOR PHYSICS

Experimental Neutron Thermalisation

By P. A. Egelstaff and M. J. Poole. With a Mathematical Appendix by P. Schofield. (International Series of Monographs in Nuclear Energy, Vol. 106.) Pp. ix + 399. (Pergamon: Oxford, London and New York, September 1969.) 130*s*; $17.50.

THE scale of investment in nuclear power already dwarfs that in almost every other science based industry, and will presumably increase very rapidly for some decades, unless there is a sudden breakthrough in fusion technology. It is estimated that the market for uranium ore alone will be worth more than $1,000 million yearly by the mid-1970s, and there is an obvious incentive to make the best use of this valuable material. On this scale, even apparently insignificant improvements in the accuracy of reactor core physics calculations can lead to very large financial savings. Basic to these calculations is an understanding of the physics of neutron scattering and thermalization in the most common reactor materials, and in this area considerable progress has been made in recent years, chiefly by groups working at the large national laboratories. Much of this work has been presented at IAEA conferences or in report form, and perhaps the major contribution of this book is to bring together the most significant elements of this material in a more readily digestible form. The authors have been responsible for many important advances in this field over a number of years, and the book can be regarded as an authoritative statement of progress up to 1968, which is the latest date on the referenced material.

The first three chapters are devoted to a rather brief development of the basic theory of neutron scattering in condensed matter, and to the analysis of theoretical models for the spectral density in various moderator materials. Chapter four covers the experimental techniques available for the production of neutrons of specified energy, and for the measurement of the energies of scattered neutrons. This is followed by an account of the application of these techniques in the measurement of scattering cross-sections, and by three chapters which discuss the analysis and interpretation of the resulting data. The second half of the book is given over to a description of techniques developed for the measurement of neutron energy spectra in moderators and reactor lattices, and to a detailed review of the results obtained by these means. Some of the methods used in the calculation of neutron energy spectra are discussed at greater length in an appendix.

In general the book is well written and the material is presented and discussed in logical sequence. It will undoubtedly be of considerable value to every worker in the field and to anybody involved with reactor physics calculations. It could also be recommended to students coming fresh to the subject, with the reservation that they should seek elsewhere for the material covered in chapters one and four.

At first glance the book is well produced, but detailed reading of some sections reveals an unfortunate incidence of misprints; where these arise in the text the meaning is usually clear, but in several instances the errors in equations lead to undue confusion in reading the associated text. In any future edition of this useful book some attention might be given to proof-reading.

The neutron chopper depicted schematically on the jacket would seem to have some useful properties.

J. SCOBIE

GRAVITATIONAL OBSERVATIONS

Gravitation and the Universe

By Robert H. Dicke. (Jayne Lectures for 1969. Memoirs of the American Philosophical Society, Vol. 78.) Pp. xvi + 82. (American Philosophical Society: Philadelphia, 1970.) $2.50.

BEFORE 1969, the last occasion on which the Jayne Lectures of the American Philosophical Society were devoted to a topic in the physical sciences was in 1965, when Professor William A. Fowler gave his notable series of talks on nuclear astrophysics. Last year, it was again the turn of astronomy to provide the subject for the Jayne Lectures, when Professor R. H. Dicke discussed "Gravitation and the Universe", and his three lectures have now been published in monograph form. Dicke discusses the problems inherent in any attempt to distinguish between Einstein's theory of gravitation and the scalar–tensor theory of which he is a major proponent, the first lecture being devoted to the Eötvös experiment as an example of laboratory tests of both theories, the second discussing the possible contribution of solar oblateness to the many perturbations of Mercury's orbit, and the third dealing with cosmological effects and the significance of the "3° K" background radiation.

Laboratory tests cannot distinguish between the predicted effects of the two theories, but provide solid evidence in support of both. Also, as Dicke sums up, although the scalar–tensor theory could provide a key to our interpretation of observations of the "cosmic fireball" if there were other strong evidence in favour of the theory, it is doubtful that "observations of the cosmic fireball will ever supply the essential element needed for the confirmation or rejection of the scalar–tensor theory". The issue here is further confused by the recent observations (not discussed by Dicke) suggesting that the background radiation may be "grey" rather than "black", so that the question of solar oblateness and the real magnitude of the residue in the perihelion rotation of Mercury is at present of overriding significance as a test to distinguish between the two theories, although sufficiently accurate measurements of the gravitational bending of light may shortly provide an independent test. There is no attempt to gloss over the difficulties involved with each kind of experiment, although naturally Dicke is not an unbiased reporter, and this book provides a useful insight into the way in which cosmologists, solar astronomers and laboratory physicists can all contribute to an investigation of the gravitational interaction.

Suitable for the non-specialist or student rather than those working directly on these problems, the monograph is excellent value, being lavishly illustrated and generally well produced apart from a few minor misprints.

JOHN GRIBBIN

Obituaries

Dr D. F. Martyn

THE death of David Forbes Martyn on March 6 has removed a pioneer of research on the upper atmosphere and a leader of Australian science. He was born on June 17, 1906, in Cambuslang, Scotland, the son of Dr Somerville Martyn. His early education at Plymouth College and Allan Glen's School was followed by a period at the Royal College of Science, London, from which he graduated BSc, ARCSc, in 1926. He took his PhD in 1929, and in 1936 was awarded the degree of DSc by the University of London. He was Houldsworth Fellow of the University of Glasgow from 1927 to 1929.

In 1929 Martyn moved to Australia, to a post with the Radio Research Board, which was attached to Mount Stromlo Observatory, Canberra. This was later incorporated into the Commonwealth Scientific and Industrial Research Organization (CSIRO), and at the time of his death he was chief officer of the CSIRO Upper Atmosphere Section.

In 1936 Martyn reconciled a number of apparently conflicting observational results by providing a remarkable model of the upper atmosphere. He showed that, although there is a warmer region above the stratosphere, the temperature must drop again to a very low value (about 160 K) at 82 km and then rise to very high values (about 1,000 K) at heights above 250 km. He also concluded that the gases at such heights were fully mixed by winds and so consisted mainly of nitrogen. These results have since been amply confirmed.

Before the Second World War he also collaborated in a number of ionospheric problems including the basis of the theory of interaction of radio waves and the polarization of downcoming radio waves. After the war he continued these studies, discovering the existence of large semidiurnal lunar oscillations in the height and electron density of the ionospheric F region. He showed that the oscillations are the result, not of local winds, but of electrodynamic drifts caused by electric fields generated in the lower "dynamo" or E region and communicated upwards along the highly conducting geomagnetic field lines. Martyn was the first to give a clear outline of the morphology of ionospheric "storms" and to show that they develop almost simultaneously all over the globe according to a relatively simple pattern when expressed in geomagnetic time.

In 1948 Martyn attacked the long outstanding problem of the dynamo theory of the daily geomagnetic variations. The known tidal velocities and electrical conductivity in the ionosphere are inadequate to explain these variations. Martyn suggested that Hall conductivity might remove the discrepancy and later, in collaboration, developed a full theory. He contributed also to the theory of radio emission from the Sun.

Immediately before the Second World War, Martyn was sent to Britain by the Commonwealth Government to learn the secrets of radar. On his return he became the first chief of the Radiophysics Laboratory of CSIRO, where he started radar development in Australia. As director of operational research for the Australian fighting services from 1942 to 1944 he took an important part in the war in the Pacific.

Martyn's greatest contribution to science in Australia was probably his role in the foundation of the Australian Academy of Science. As one of the Fellows of the Royal Society resident in Australia who initiated and carried through this difficult task, he worked indefatigably, with great patience and tact, never allowing the many personal and practical difficulties to dampen his enthusiasm. It was Martyn who as first Secretary (Physical Sciences) produced from the proposals made by his colleagues the statutes and rules, on the basis of which a Royal Charter was presented to the founding fellows by the Queen, during her visit to Canberra in 1954. He was president when he died.

Martyn was chairman of the Australian National Committee for Space Research and the United Nations Scientific and Technical Committee on the Peaceful Uses of Outer Space. He served with URSI as chairman of the Radio Astronomy Commission and as chairman of the Ionosphere Commission. He was awarded the Chree Medal of the Physical Society of London in 1954, the Lyle Medal of the Australian National Research Council in 1947, the Sidey Medal of the Royal Society of New Zealand in 1947 and the Burfitt Medal of the Royal Society of NSW in 1950, and his contributions to science were recognized by other learned societies. He was elected to the Royal Society in 1950.

David Martyn was a man of strong convictions, held tenaciously and often pugnaciously, so that his friendships and his enmities brought him troubles and anxieties. He fought fearlessly for integrity and high standards in science. He is survived by his widow whom he married in 1944.

Correspondence

Dust-up in Nevada

SIR,—*Nature* states[1] that Emiliani *et al.*[2] did not prove a causal relationship between underground nuclear explosions and earthquakes. If a proof was not provided, it is not for the reasons advanced by *Nature* but for the much more fundamental reason that you cannot prove anything by statistics.

In the paper in question[2], it was stated that significant earthquake activity increase took place in annuli up to 860 km away from testpoint. Anderson *et al.*[3] wrote that these results "would be important" if a significant earthquake activity increase could be shown between 400 and 860 km from testpoint. In their reply, Emiliani *et al.*[4] showed just that. Therefore, the results obtained should be important, at least by CalTech standards. However, it is within the realm of statistical possibility that analysis of data across a much greater time interval (largely as yet not available) might lead to different or even opposite conclusions.

Nature is correct in claiming that the reaction of nine distinguished seismologists to our paper is more psychological than geophysical. The overreaction is explained by the fact that the authors of the paper are not seismologists and have never contributed to this field, thus making their contribution of (presumably) important results vastly annoying to some. As to priorities, the first published suggestion to use underground nuclear explosions to control earthquakes is due to Brune and Pomeroy[6]. This idea, however, appears to have been previously the object of informal discussion and speculation[5] to which, alas, Emiliani *et al.* were not invited (personal communication).

Yours faithfully,

C. EMILIANI

School of Marine and Atmospheric Sciences,
University of Miami,
Miami, Florida 33149.

[1] *Nature*, **225**, 1099 (1970).

[2] Emiliani, C., Harrison, C. G. A., and Swanson, M., *Science*, **165**, 1255 (1969).

[3] Anderson, D. L., Archambeau, C. B., Brune, J. N., Richter, C. F., and Smith, S. W., *Science*, **167**, 1012 (1970).

[4] Emiliani, C., Harrison, C. G. A., and Swanson, M., *Science*, **167**, 1013 (1970).

[5] *Time*, p. 82 (October 17, 1969).

[6] Brune, J. N., and Pomeroy, P. W., *J. Geophys. Res.*, **68**, 5020 (1963).

Coping with Oil Slicks

SIR,—In his letter about oil slicks (*Nature*, **226**, 573; 1970) Mr B. Pounder advocated taking away from the Board of Trade responsibility for dealing with oil pollution at sea because of the Board's alleged impotence in the face of the technical problems involved.

This responsibility was given to the Board of Trade last summer because it was the civil department in the best position to undertake it. The major work of planning that was required all round the coast is now well advanced and the Board is procuring special equipment to enable it to deal with serious spillages. The Board does not stand alone in this work, but can call upon the help of the Royal Navy and the Royal Air Force and on scientific and technical advice from Government laboratories, especially the Warren Spring Laboratory.

However, this does not mean that we shall be able to clear up every oil slick at sea. The obstacle to this is not technical but economic. The slicks reported round our coasts are numerous, and to attempt to investigate and deal with each one would be disproportionately costly in money and resources. Our planning must therefore concentrate on serious spillages threatening the coast.

The real answer to the threat to seabirds from continual relatively minor oil pollution is to prevent the oil from getting on to the sea in the first place by bringing in a more readily enforceable set of restrictions on discharges from ships. This has to be an international matter, and the United Kingdom has taken the lead in securing agreement to amendments to the International Convention on Prevention of Pollution of the Sea by Oil 1954 for this very purpose. The Oil in Navigable Waters Bill that was introduced into Parliament on May 12 is intended to enable the government to accept these amendments formally.

Yours faithfully,

MICHAEL YOUNG

Board of Trade,
1 Victoria Street,
London SW1.

Prowess of P's

SIR,—Dr Weale (*Nature*, **226**, 195; 1970) suggests that there may be a marginal disadvantage in having a name beginning with a letter coming towards the end of the alphabet. A few years ago I noticed that the midpoint in the membership lists of the Biochemical and Nutrition Societies came among the Ls whereas in the Royal Society and National Academy of Sciences lists it was in the Ks. This suggested a slight forward drift in organizations recruited on the basis of apparent merit. It would be interesting if someone with more patience would conduct a more comprehensive survey.

Yours faithfully,

N. W. PIRIE

42 Leyton Road,
Harpenden, Hertfordshire.

Tektites tracked to Toba

SIR,—D. H. McColl and G. E. Williams are to be congratulated on their excellent observation[1] concerning the pattern of tektite infall in Australia. Making the assumptions that each line of infalling tektites was ejected from its own aperture of a cryptovolcanic structure, with velocities between 8 and 8·5 km/s, with azimuths of 135° and 150°, and with angles of elevation of approximately 75°, brings the intersection point of the infall lines to Lake Toba, Sumatra, after consideration of the effect of the Earth's rotation.

Lake Toba is considered a volcano-tectonic sink of the Pleistocene age[2], and is as unlikely a cryptovolcanic structure as one could hope to nominate. I should like, however, to suggest that its age be more accurately determined and that the area be investigated for the presence of coesite and shattercones.

Yours faithfully,

C. W. KAYSING

2244 Sunset Crest Drive,
Los Angeles,
California 90046.

[1] McColl, D. H., *Nature*, **226**, 154 (1970).

[2] Rittman, A., *Volcanoes* (John Wiley and Sons, 1962).

Announcements

University News

Dr Paul A. Marks, acting dean of the College of Physicians and Surgeons, has been appointed dean of the Faculty of Medicine and vice-president for medical affairs at **Columbia University,** in succession to Dr H. Houston Merritt.

Professor E. N. Hiebert, University of Wisconsin, has been appointed professor of the history of science at **Harvard University.**

A Research Unit for Instrument Physics has been established in the Department of Applied Physical Sciences, University of Reading, under the supervision of Professor P. B. Fellgett. **Dr G. F. Reynolds,** director of research and development, Shandon Scientific Company Limited, has been appointed head of the new Unit, which will be concerned primarily with industrial collaboration and with industrial research and consultancy. At first, the Unit will be supported by the University Grants Committee, but it is hoped that the fees for projects undertaken will enable it to become self-supporting. Work is already under way on problems concerning meteorological instruments, machine tools, and plastics optical systems and components, and suggestions for further projects will be welcome. Further details about the Unit can be obtained from Dr G. F. Reynolds, Research Unit for Instrument Physics, Department of Applied Physical Sciences, University of Reading, Whiteknights, Reading RG6 2AY, Berkshire.

Appointments

Dr Anna M. MacLeod, Heriot-Watt University, Edinburgh, has been made the first woman president of the Institute of Brewing.

Professor Albert B. Sabin was formally installed as **President of the Weizmann Institute of Science** on May 4.

Mr A. G. Beynon, director of Veterinary Field Services, has been appointed **Chief Veterinary Officer of the Ministry of Agriculture, Fisheries and Food,** in succession to Mr J. Reid.

Mr Frank O'Hara, Royal Aircraft Establishment, Bedford, has been appointed **Director-General of Civil Aircraft and General Services Research Development in the Ministry of Technology,** in succession to Mr D. E. Morris.

Miscellaneous

The **Experimental Pathology Club prize** for 1970 has been awarded to **Mr R. A. Little,** MRC Toxicology Unit, for his essay "The Effect of Age on the Response to Injury".

The **Spearman medal** of the British Psychological Society has been awarded to **Dr K. J. Connolly,** University of Sheffield, for his research on the genetics of behaviour and the development of skill in children.

The Council of the Royal Society has awarded a **Bruno Mendel travelling fellowship** to **Miss R. Ashkenazi,** Hebrew University—Hadassah Medical School, to enable her to work for one year at the ARC Institute of Animal Physiology, Babraham.

Dr A. R. C. Westwood, deputy director of the Research Institute for Advanced Studies, Martin Marietta Corporation, has been awarded a gold medal and a prize of £100 by the administrators of the Sir George Beilby Memorial Fund, for his work in materials science.

ERRATUM. The following corrections should be made in the article "Increase in Antiviral Activity of Polynucleotides by Thermal Activation" by E. de Clercq, R. D. Wells and T. C. Merigan (*Nature*, **226**, 364; 1970): The second sentence of the first paragraph should read "The thermal activating effect . . . was most marked with the alternating polyribonucleotides such as poly (A–U) and poly (I–C)". The second footnote in Table 1 should read "†Minimal Eagle's medium (MEM), *p*H 7·0–7·5 . . . ". In the paragraph following Table 1, the first sentence should read "Preincubation at 37° C, compared with 0° C, rendered all polynucleotides, including poly I·poly C, significantly more active in reducing virus plaque formation in human cells, when the incubation was carried out in MEM (Table 1)", and the sentence beginning on line 16 of that paragraph should read "Addition of 10^{-3} M EDTA following the 37° C incubation step or rapid cooling to 0° C (by immersion in solid CO_2–ethanol) did not reverse the activating effect . . . ".

International Meetings

June 3–4, **Launching the Corporate Plan,** Brussels (Administrative Secretary, European Society of Corporate and Strategic Planners, 71 rue de la Loi, B-1040 Brussels, Belgium).

June 7–9, **Computer Applications in the Earth Sciences,** Lawrence (R. F. Treece, University Extension, University of Kansas, Lawrence, Kansas 66044, USA).

June 9–11, **Electrical Interference in Instrumentation,** London (The Manager, Conference Department, IEE, Savoy Place, London WC2R OBL).

June 10, **Biochemistry of Photosynthesis,** London (Dr D. O. Hall, King's College, 68 Half Moon Lane, London SE24).

June 16–19, **Hospital Equipment Medical Engineering and Services Exhibition,** London (Posner-Cox Public Relations, 102 Charing Cross Road, London WC2).

June 18, **Animal Viruses as Genetic Modifiers of the Cell,** London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1).

June 29–July 2, **Book Indexing** (training course), London (Mr L. M. Harrod, c/o School of Librarianship, North Western Polytechnic, 207–225 Essex Road, London N1).

July 5–6, **Liquid Scintillation Counting for Radioisotope Measurement,** San Francisco (C. T. Peng, School of Pharmacy, University of California, San Francisco, California 94122, USA).

July 6–10, **Microwave Spectroscopy,** Bangor (Professor J. Sheridan, Department of Chemistry, University College of North Wales, Bangor, Caernarvonshire).

July 7–9, **Scanning Electron Microscopy in Materials Science,** Newcastle upon Tyne (E. H. Boult, School of Chemistry, The University, Newcastle upon Tyne NE1 7RU).

July 25–30, **Applied Psychology,** Liège (EDITEST, rue Général Capiaumont, 94–1040 Bruxelles, Belgium).

August 5–7, **Applications of X-ray Analysis,** Denver (Metallurgy and Materials Science Department, Denver

Research Institute, University of Denver, Denver, Colorado 80210, USA).

August 18–27, **General Assembly of the International Astronomical Union,** Brighton (Press Office, Science Research Council, State House, High Holborn, London WC1R 4TA).

September 7–9, **Ion Implantation,** Reading (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

September 13–15, **Science and Technology of Metal Materials,** Tremezzo (Fondazione "Guido Donegani", Accademia Nazionale dei Lincei, Via della Lungara 10, 00165 Roma, Italy).

September 15–18, **Gas Discharges,** London (Manager, Conference Department, IEE, Savoy Place, London WC2R OBL).

September 27–October 2, **Cell Walls and Cell Membranes,** Ashford, Kent (Executive Secretary, 7 Warwick Court, London WC1R 5DP).

September 29–October 1, **Autumn Meeting of the Chemical Society,** London (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V OBN).

October 28–29, **Radiation and Isotope Techniques in the Building Industry,** Brussels (EURISOTOP Office, Commission of the European Communities, Berlaymont Building, 200 rue de la Loi, B-1040 Brussels, Belgium).

November 10–12, **Laboratory Automation,** London (Conference Registrar, Institution of Electronic and Radio Engineers, 8–9 Bedford Square, London WC1B 3RG).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Flat in London for 6 months from July 1, 1970, by visiting Australian chemist and wife. Convenient for access to Imperial College. Central heating preferred. Please contact Dr M. J. Gallagher, East Chemistry Building, University of Illinois, Urbana, Illinois 61801, USA.

Wanted: By Dutch lecturer with four children, furnished house, near either the Torry Research Station, Aberdeen, or Unilever Research Laboratories, Bedford. From July to October inclusive. State whether crockery and blankets can be provided. Please contact Professor D. G. Hawkridge, Open University, Bletchley, Buckinghamshire, before June 21.

Wanted: Furnished 3 bedroom house or flat in London or environs, with easy access to Chelsea College, from September 1, 1970, for approximately one year. Please contact Professor John W. Martin, Department of Chemistry, Bridgewater College, Bridgewater, Virginia 22812, USA.

Vacant: Modern detached house in Hassocks to let from mid-July for one year. Three bedrooms, lounge, dining room, central heating. Close to Brighton and the University of Sussex. London one hour by rail. Please contact Dr A. D. Baddeley, 47 Wilmington Close, Hassocks, Sussex.

British Diary

Tuesday, May 26

Excavations at Marlik (5.45 p.m.) Professor E. O. Negahban (Tehran) University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Vibration Theory I (6 p.m.) Professor B. Fraeijs de Veubeke, University of London, at Queen Mary College, London E1.

Wednesday, May 27

Eighth Annual Tribology Group Convention (three days) Institution of Mechanical Engineers, Tribology Group, at Brighton.

Electronic Circuits for Medical Instrumentation (2 p.m. discussion meeting) Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical and Biological Electronics Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Human Pituitary Diseases (5.30 p.m.) Professor Russell Fraser, University of London, at the Royal College of Physicians, St. Andrew's Place, London NW1. (Sandoz Foundation Lecture in Endocrinology.)

The Architect and Power Engineering (5.30 p.m.) The Rt. Hon. The Lord Holford of Kemp Town, Institution of Electrical Engineers, at Savoy Place, London WC2.

Thursday, May 28

Annual General Meeting followed by Technical Films (5.30 p.m.) Institution of Electrical Engineers, at Savoy Place, London WC2.

Challenge of the Environment to Architecture and Building Science (5.30 p.m.) Professor Albert G. H. Dietz (Massachusetts), University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Computer Aids in Mechanical Engineering Design and Manufacture (9.30 a.m. discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

On Line Electrochemical Sensors (6.30 p.m.) Society for Analytical Chemistry, Electroanalytical and the Automatic Methods Groups, at the Leicester Lounge, 44 Glasshouse Street, London W1.

Regional Variation in Indian Ocean Coral Reefs (two-day symposium) Royal Society and the Zoological Society of London, in the Meeting Room of the Zoological Society of London, Regent's Park, London NW1.

Virtues and Vices of Pressure Testing (6 p.m. discussion meeting) Institution of Mechanical Engineers, Applied Mechanics Group, at 1 Birdcage Walk, London SW1.

Friday, May 29

Developmental and Diagnostic Pathology of Endocrine Polypeptide Tumours (11 a.m.) Professor A. G. E. Pearse, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Monday, June 1

Immunological Tolerance: Its Possible Role in Organ Transplantation (5 p.m.) Professor L. Brent, University of London, at the Wright-Fleming Institute, St. Mary's Hospital Medical School, Norfolk Place, London W2.

Reports and Publications

(not included in the monthly Books Supplement)

Other Countries

Museum of Comparative Zoology, Harvard University. Breviora. No. 334: Postlarval *Sudis* (Pisces: Paralepididae) in the Atlantic Ocean. By David L. Shores. Pp. 14. No. 335: Two New Late Cretaceous Fishes from Montana and Wyoming. By Richard Estes. Pp. 15. No. 336: The Status of the East African Elephant *Archidiskodon exoptatus* Dietrich 1942. By Vincent J. Maglio. Pp. 25. No. 337: Lower Vertebrates from the Late Cretaceous Hell Creek Formation, McCone County, Montana. By Richard Estes, Paul Berberian and Charles A. M. Meszoely. Pp. 33. No. 338: A New Frog of the Genus *Discodeles* (Ranidae) from Guadalcanal Island. By T. Preston Webster. Pp. 9. No. 339: The Genus *Stenoonops* (Araneae, Oonopidae) in Panama and the West Indies. By Arthur M. Chickering. Pp. 35. No. 340: Distribution of the Centrolophid Fish *Schedophilus pemarco*, with Notes on Its Biology. By Richard L. Haedrich and Fernando Cervigon. Pp. 9. No. 341: Four New Species of Elephantidae from the Plio-Pleistocene of Northwestern Kenya. By Vincent J. Maglio. Pp. 43. Bulletin of the Museum of Comparative Zoology, Vol. 138, No. 7: An Evolutionary Microcosm—Pleistocene and Recent History of the Land Snail *P.* (*Poecilozonites*) in Bermuda. By Stephen Jay Gould. Pp. 407–532. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1969.) [242

US Department of the Interior: Geological Survey. Bulletin 1281: Geology Along the Taylor Highway, Alaska. By Helen L. Foster and Terry E. C. Keith. Pp. viii + 36 + plate 1. Water-Supply Paper 1663-F: Ground-Water Resources of the Lambayeque Valley, Department of Lambayeque, Northern Peru. By Stuart L. Schoff and Juan Luis Sayan M. Pp. vi + 77 + plates 1–3. Professional Paper 643-C: *Pseudoleperditia* Schneider, 1956 (Ostracoda, Crustacea), an Early Mississippian Genus from Southwestern Nevada. By I. G. Sohn. Pp. iii + 6 + plate 1. $0.30. Professional Paper 680-c: Larger Foraminifera from Deep Drill Holes on Midway Atoll. Pp. iii + 15 + plates 1–5. $0.45. (Washington, DC: Government Printing Office, 1969.) [242

Sveriges Geologiska Undersökning. Ser. C Nr. 638: Blockstudier i Flygbilder: En Metodundersökning av Möjligheten att Kartera Markytans Blockhalt fran Flygbilder. By Leid Wastenson. Pp. 95 + 2 plates. Ser. C. Nr. 639: Conodont Zones in the Ludlovian of Gotland and a Correlation with Great Britain. By Lars E. Fahraeus. Pp. 33 (2 plates). Ser. C. No. 641:

Elements in Microphotography with Ordinary Light. By K. E. Samielsson. Pp. 35 (8 plates). Ser. C. Nr. 642: Fossil Diatoms under the Scanning Electron Microscope—a Preliminary Report. By Urve Miller. Pp. 65 (25 plates). Ser. C. Nr. 644: Caledonian Geology of Northern Jämtland—Southern Västerbotten. By Ebbe Zachrisson. Pp. 33 + 1 plate. Ser. C. Nr. 648: Problems of the So-called Rogen Moraine. By Jan Lundqvist. Pp. 32. (Stockholm: Sveriges Geologiska Undersökning, 1969.) [252

Norsk Polarinstitutt. Arbok 1968. Pp. 138. (Oslo: Norsk Polarinstitutt, 1970.) [252

Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series, No. 25: Lands of the Adelaide—Alligator Area, Northern Territory. Comprising papers by R. Story, M. A. J. Williams, A. D. L. Hooper, R. E. O'Ferrall and J. R. McAlpine. Pp. 153. (Melbourne, CSIRO, 1969.) [252

Annual Report of the Experiment Station of the South African Sugar Association, 1968/1969. Pp. 52. (Mount Edgecome, Natal: Experiment Station of the South African Sugar Association, 1970.) [252

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wheat Research Unit, 1968/1969. Pp. 17. (Ryde, NSW: CSIRO, 1970.) [252

New Zealand: Department of Health. Environmental Radioactivity in New Zealand and Measurements on Samples from Fiji and Rarotonga, Qtly. Report, July–September, 1969. Pp. 11. (Christchurch: National Radiation Laboratory, 1969.) [23

European Defence Co-operation and NATO. By Walter Schütze. (The Atlantic Papers, No. 3.) Pp. 60. (Paris: The Atlantic Institute, 1969.) \$1.40; 10*s*; 6 francs; 5 DM. [23

République Démocratique du Congo: Office National de la Recherche et du Développement. Publications de l'Institut pour l'Étude Agronomique du Congo. Carte des Sols et de la Végétation du Congo, du Rwanda et du Burundi. 25. Bas-Congo. B: Notice Explicative de la Carte de la Végétation. Par P. Compère. Pp. 35. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1970.) [23

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 183: Geology of Ordovician to Pennsylvanian Rocks, M'Clintock Inlet, North Coast of Ellesmere Island, Canadian Arctic Archipelago. By H. P. Trettin. Pp. 93. \$2. Paper 68–25: Subsurface Geology, Lower Mackenzie River and Anderson River Area, District of Mackenzie. By E. J. Tassonyi. Pp. x + 207. \$3.50. Paper 69–34: Distribution of Open-System Pingos in Central Yukon Territory with Respect to Glacial Limits. By O. L. Hughes. Pp. v + 8. \$1.50. Paper 69–39: Attributes of Stromatolites. By H. J. Hofmann. Pp. 58. \$2. (Ottawa: Queen's Printer, 1969.) [33

Fisheries Research Board of Canada. Bulletin 172: The Harbour Seal in British Columbia. By Michael A. Bigg. Pp. viii + 33. (Ottawa: Queen's Printer, 1969.) \$2. [33

Czechoslovak Academy of Sciences, Institute of Microbiology. Annual Report of the Laboratory of Algology for the year 1968. Pp. 205. (Trebon: Czechoslovak Academy of Sciences, Institute of Microbiology, 1969.) [33

Deutsche Akademie der Wissenschaften zu Berlin. Arbeiten aus dem Geodätischen Institut Potsdam. Nr. 24: Bestimmung Kurzperiodischer Fehler bei Kreisteilungen. Von Hans Weise. Pp. 45. Nr. 25: Zufällige und Systematische Fehler in Geodätisch–Astronomischen Zeitbestimmungen. Von Ernst Buschmann. Pp. 85. Nr. 26 : Fehlertheoretische Untersuchungen zur Kosmischen Triangulation. Von Dietrich Schoeps. Pp. 70. Nr. 27: Uber die Ursache der Alterungserscheinung an Schwingsquarzen. Von Victor Kroitzsch. Pp. 109. (Potsdam, DDR.: Deutsche Akademie der Wissenschaften zu Berlin, 1969.) [33

The Nucleus: Annual Review of the Science Foundation for Physics and the School of Physics within the University of Sydney. Pp. 80. (Sydney: The University, 1970.) [43

Cold Spring Harbor Laboratory of Quantitative Biology. Annual Report 1969. Pp. 39. (Cold Spring Harbor, L.I.: Cold Spring Harbor Laboratory of Quantitative Biology, 1970.) [43

Memorie dell' Istituto Lombardo–Accademia di Scienze e Lettere. Classe de Scienze, Matematiche e Naturali. Vol. XXVI, Memoria 1: Il Clima della Valtellina in Relazione alla Difesa del Suolo. By Severino Belloni. Pp. 75. (Milano: Istituto Lombardo di Scienze e Lettere, 1969.) [43

Illinois Natural History Survey Bulletin, Vol. 30, Article 1: Largemouth Bass and Other Fishes in Ridge Lake, Illinois, 1941–1963. Pp. 67. (Urbana, Ill.: Department of Registration and Education, Natural History Survey Division, 1969.) [63

Field Museum of Natural History. Fieldiana: Anthropology. Vol. 59 (January 16, 1970): Kijik—an Historic Tanaina Indian Settlement. By James W. Vanstone and Joan B. Townsend. Pp. 202. (Chicago: Field Museum of Natural History, 1970.) [63

CERN European Organization for Nuclear Research. Proceedings of the 1969 CERN School of Physics, Leysin, 31 August–13 September, 1969. Pp. 208. (Geneva: CERN, 1969.) [63

US Department of the Interior: Geological Survey. Bulletin 1294-E: The Bates Mountain Tuff in Northern Nye County, Nevada. By K. A. Sargent and E. H. McKee. Pp. iii + 12. \$0.10. Bulletin 1312-J: Preliminary Geologic Investigations in the Kanuti River Region, Alaska. By William W. Patton, Jr., and Thomas P. Miller. Pp. iii + 10. \$0.15. Bulletin 1314-A: Geochemical Investigations of Some Black Shales and Associated Rocks. By James D. Vine and Elizabeth B. Tourtelot. Pp. iv + 42. \$0.30. Water-Supply Paper 1901: Surface Water Supply of the United States, 1961–65. Part 1: North Atlantic Slope Basins. Vol. 1: Basins from Maine to Connecticut. Pp. xi + 1027. (Washington, DC: Government Printing Office, 1969 and 1970.) [93

Smithsonian Contributions ot Paleobiology. No. 2: A New Occurrence of Paleocene Mammals in the Evanston Formation, Southwestern Wyoming. By C. Lewis Gazin. Pp. 12 + 3 plates. \$0.45. Smithsonian Contributions to Zoology. No. 36: A Review of the Genus *Harpiosquilla* (Crustacea, Stomatopoda), with Descriptions of Three New Species. By Raymond B. Manning. Pp. iii + 41. \$0.50. No. 41: Revision of the Aphroditoid Polychaetes of the Family Eulepthidae Chamberlin (= Eulepidinae Darboux; = Pareulepidae Hartman). By Marian H. Pettibone. Pp. 44. No. 42: Review of Some Little Known Genera of Serpulidae (Annelida: Polychaeta). By Helmut E. Zibrowius. Pp. 22. \$0.35. (Washington, DC: Government Printing Office, 1969.) [93

US Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Supplement 16, Part 6: Parasite—Subject Catalogue—Subject Headings and Treatment. By Dorothy B. Segal, Shirley M. Humphrey, Shirley J. Edwards and Margie D. Kirby. Pp. iii + 138. \$1.25. Supplement 17, Part 4: Parasite Subject Catalogue. Parasites: Nematoda and Acanthocephala. By Dorothy B. Segal, Judith M. Humphrey, Shirley J. Edwards and Margie D. Kirby. Pp. iii + 269. Supplement 17, Part 5: Parasite Subject Catalogue. Parasites: Arthropoda and Miscellaneous Phyla. By Dorothy B. Segal, Judith M. Humphrye, Shirley J. Edwards and Margie D. Kirby. Pp. v + 290. \$2.25. (Washington, DC: Government Printing Office, 1969.) [93

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US National Museum Bulletin No. 277: A Revision of the Scenopinidae (Diptera) of the World. By L. P. Kelsey. Pp. v + 335. (Washington, DC: Smithsonian Institution Press, 1969. For sale by US Government Printing Office.) \$2. [93

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Perspectives in Oceanography, 1968. (Intergovernmental Oceanographic Commission Technical Series, No. 6.) Pp. 90. (Paris: Unesco; London: HMSO, 1969.) 12 francs; 18*s*; \$3. [93

Transactions of the American Philosophical Society, New Series, Vol. 60, Part 1: The Paleolithic Cultures of Singhbhum. By Asok K. Ghosh. Pp. 68 (42 plates). (Philadelphia: American Philosophical Society, 1970.) \$2.50. [93

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Forest Products, 1968/1969. Pp. 78. (Melbourne: CSIRO, 1970.) [103

Ciba Agrochemicals Technical Monograph No. 2: Waterless Spraying from the Air. Pp. 28. (Basle: Ciba, Ltd., 1970.) [103

Bulletin of the Florida State Museum—Biological Sciences. Vol. 14, No. 3: The Cottonmouth Moccasin on Sea Horse Key, Florida. By Charles H. Wharton. Pp. 227–272. \$0.80. Vol. 14, No. 4: The Pliocene Canidae of Florida. By S. David Webb. Pp. 273–308. \$0.70. (Gainesville: Florida States Museum, 1969.) [103

Annual Report of the Geophysical Institute, University of Alaska, 1968/1969. Pp. 111. (College, Alaska: Geophysical Institute, 1970.) [103

Occasional Papers of the California Academy of Sciences. No. 79: A Systematic Review of the Scorpion fishes of the Atlantic Ocean (Pisces: Scorpaenidae). By William N. Eschmeyer. Pp. iv + 143. Proceedings of the California Academy of Sciences, Fourth Series. Vol. 37, No. 3: Late Pliocene Mollusks from San Francisco Peninsula, California, and their Paleogeographic Significance. By Warren O. Addicott. Pp. 57–93 + 4 plates. Vol. 37, No. 4: The Biology of Storm Petrels in the Galapagos Islands. By M. P. Harris. Pp. 95–166. (San Francisco: California Academy of Sciences, 1969.) [103

Annual Report on the Forest Administration of Western Nigeria, 1965/1966. Pp. v + 32. (Ibadan: Ministry of Agriculture and Natural Resources, 1969.) 3*s* 6*d*. [103

Smithsonian Contributions to Zoology, No. 35: Cranial and Bacular Variation in Populations of Spiny Rats of the Genus *Proechimys* (Rodentia: Echimyidae) from South America. By Robert E. Martin. Pp. 19. (Washington: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) \$0.35. [103

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UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF CHEMISTRY

Applications are invited for two POSTGRADUATE awards (S.R.C. rates plus fees) for research in the field of organophosphorus chemistry. Candidates would be expected to register for the degree of Ph.D. (University of London) and should have, or expect to have, a good B.Sc. degree or equivalent qualifications. The Department is fully equipped with modern instruments (including n.m.r. and e.s.r.) and attendance at postgraduate summer courses would be supported and encouraged.

Applications (including curriculum vitae and the names of two referees), should be sent, as soon as possible, to Dr. C. D. Hall (c/o Professor D. H. Hey, F.R.S.), Department of Chemistry, King's College London, Strand, London, WC2R 2LS.

(1934)

UNIVERSITY OF SOUTHAMPTON

INSTITUTE OF SOUND AND VIBRATION RESEARCH

LECTURESHIP IN STRUCTURAL DYNAMICS

Applications are invited for the post of Lecturer in the Institute of Sound and Vibration Research. Applicants should have experience and research interests in the field of structural dynamics. The person appointed will be expected to assist in the undergraduate and postgraduate teaching in the Institute and also to develop research work in one or more aspects of structural dynamics. Salary scale: £1,585 to £2,850 with a merit bar at £2,045.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton SO9 5NH, to whom applications (seven copies from United Kingdom applicants) should be sent by not later than May 30, 1970, quoting ref.: N. (1922)

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Applications to Dr. Mills, Courtauld Institute, The Middlesex Hospital Medical School, London, W1P 5PR. (1965)

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RESEARCH ASSISTANT IN DENTAL BIOCHEMISTRY

Applications are invited from graduates with an Honours degree in Biochemistry or related subject for the post of Research Assistant in Dental Biochemistry. The successful applicant will perform research into the enzymology of carbohydrate metabolism by bacteria. The project will be partially financed by an award from the Medical Research Council and may provide the opportunity for a suitably qualified candidate to register for a higher degree. Salary scale—if postdoctoral £1,240 to £1,470 per annum, otherwise £800 to £1,250 per annum. F.S.S.U.

Applications (three copies), should be lodged with the undersigned, from whom further particulars may be obtained. In reply please quote Ref. No. 5016-M.

ROBT. T. HUTCHESON,
Secretary of the University Court.

(1931)

For further Classified advertisements see page iv

AUSTRALIA

NEW SOUTH WALES DEPARTMENT OF AGRICULTURE

Senior Research Officer

(WHEAT BREEDING AND GENETICS)

Agricultural Research Institute, Wagga Wagga

Applications are invited for the above position.

Salary

$A6,551 Range $A7,004 per annum with progression to $A7,571 per annum subject to conditions.

Qualifications

Degree in Agricultural Science or equivalent

Experience

essential—several years experience in Wheat Breeding and/or Wheat Genetics.

Duties

To formulate and pursue a research programme in Wheat improvement in both basic and applied aspects, and to continue an existing programme in these areas.

Accommodation

Single accommodation can be supplied to male appointees at $A7–41 per week.

Appointment and Travel

the services of the appointee are required as soon as possible and applicants should be prepared to emigrate in the near future

Subject to certain conditions the successful applicant will be eligible for:

- ★ Payment of fares to Sydney.
- ★ Financial assistance towards removal and establishment expenses.
- ★ Financial assistance towards initial accommodation expenses.

For further information and application form write to the Recruitment Section, New South Wales, Government Offices, 56 Strand, London, W.C.2, where applications close on the **19th JUNE 1970**. When writing please quote reference 44/442

Hon. A. Landa, Agent General.

(2117)

KODANSHA (PUBLISHERS) LTD., TOKYO

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Kodansha, Japan's principal publisher, has an immediate opening for a full-time English Language Editor to prepare manuscripts for publication in the field of chemistry.

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Duties:	Language correction of translations from Japanese; editing and preparing manuscripts for book publication. Full responsibility.
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Written applications stating full name, place, date and year of birth, nationality, marital status, present employment, details of education, qualifications and experience, and previous acquaintance with Japan, if any, should be addressed to **Mr. Y. Haga, Dept. of Research for Scientific Edition, Kodansha Ltd., 12-21, Otowa 2-chome, Bunkyo-ku, Tokyo 112, Japan,** to arrive not later than 17 June, 1970.

(2027)

National Council for Scientific Research, Lusaka - Zambia

VACANCIES EXIST FOR PROFESSIONAL AND TECHNICAL OFFICERS AS UNDER:

PROFESSIONAL OFFICERS

1. Entomologist—Tsetse Breeding—Tsetse Research Team

Duties: To maintain and develop colonies of tsetse fly, and to carry out research investigations leading to improved fly breeding and feeding techniques. To cooperate with other members of the team in the various projects leading to the use of chemosterilization for tsetse eradication.

Qualifications: Good University Hons. Graduate in Entomology or alternatively Zoology or Biology with appropriate graduate and post graduate training or experience. Approved post graduate experience in entomology would be required, and it would be particularly advantageous if this were in the field of tsetse.

2. Ecologist—Tsetse Research Team

Duties: To investigate the relationship of tsetse populations to various components of their habitat, in connection with research into the use of chemosterilants as a means of tsetse eradication.

Qualifications: Good University Hons. Graduate in Zoology, Ecology, Entomology or other appropriate subjects. Post graduate research and/or practical experience in ecology, field entomology or related areas would be required. A knowledge of tsetse control activities would be advantageous, as would experience in the direction of field workers.

3. Field Ecologist—Unit of Tick Bionomics

Duties: To assist in field work on tick biology, host relationships and disease transmission.

Qualifications: Degree in biology, agriculture or veterinary science.

4. Microbiologist—Unit of Tick Bionomics

Duties: Biology of microbes associated with ticks, including mammal pathogenicity.

Qualifications: Biologist, Hons. degree or equivalent in Zoology. Post graduate experience of modern methods of cultivation and typing of micro-organisms. Familiarity with veterinary pathogens an advantage, but not essential.

5. Insect Physiologist—Unit of Tick Bionomics

Duties: The influence of environment on survival development and reproduction of ticks, the behaviour responses of ticks, and the biochemistry of host stimuli.

Qualifications: Hons. degree or equivalent in Biology, with post graduate experience in insect physiology. Experience of modern biochemical techniques an advantage.

TECHNICAL OFFICERS

1. Tsetse Research

Duties: To be responsible, under the direction of the Professional Officers, for the execution of the research programme. In particular the day-to-day running of the field investigations, together with the collecting and maintenance of all necessary records on tsetse populations, etc., would be a major responsibility.

Qualifications: G.C.E. 'O' levels or equivalent, together with a suitable diploma or alternatively experience and inservice training. The applicant should have experience in tsetse field investigations or closely related field projects, He should also have experience of man management, and will probably have worked in a tsetse control department. game department or similar organization.

2. Unit of Tick Bionomics

(a) One technical Officer with experience of pathological and diagnostic methods.
(b) One technical officer with entomological training. To be in charge of laboratory work on ticks.
(c) One technical officer with entomological or general parasitological training, or training in or experience of animal husbandry.

Qualifications: G.C.E. 'O' levels or equivalent, together with a suitable diploma or alternatively appropriate experience and inservice training. He should also have experience of man management.

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Closing date June 30, 1970.

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(2098)

UNIVERSITY OF DUBLIN
Trinity College

LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the above post which has been established in conjunction with the Irish Flour Millers' Association.

The lecturer, who should have experience in the chemistry or biochemistry of proteins, will be expected to interest himself in research on the chemical and nutritional aspects of flour.

Salary Scale:
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It is not expected that an appointment will be made above the Bar in the Lecturer's scale.

Marriage and children's allowances are paid and there is a non-contributory F.S.S.U.-type pension scheme.

Further particulars may be obtained from:

The Assistant Secretary (Staff),
Trinity College,
Dublin 2.

who will receive completed applications up to June 22, 1970. (2109)

AUSTRALIA

Queensland Institute of Technology

BRISBANE

Applications are invited for the following positions :

Department of Biological Science

SENIOR LECTURERS
LECTURER

Positions are available in the following fields :

Genetics—population genetics, human genetics, or microbial genetics.
Education in Biological Sciences.
Fresh-water Ecology.
Herbicides. Entomology.

Initiative in the development of applied research programmes ancillary to the courses offered is a primary concern of the Department. The Department conducts a degree level course in Biological Science.

Department of Chemistry

LECTURERS IN GEOLOGY

Positions are available in the following fields :

Sedimentology.
Stratigraphy. Palaeontology.

Suitably qualified persons with experience in other aspects of geology should apply. A three-year Associate Diploma in Applied Geology may be introduced in 1971. This will be of degree standard.

Department of Physics

SENIOR LECTURER
LECTURERS

The Senior Lecturer should have a major interest and experience in one of the fields of applied physics with a definite bias towards experimental applied physics in the areas of :

Applied Geophysics.
Materials Science. Biophysics.

The Lecturer should have interest and experience in one of the fields of applied physics.

Qualifications

Applicants should possess at least an honours degree or equivalent and should have adequate experience.

Salary Ranges

Senior Lecturer A, $A7,500 to $A8,750.
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Lecturer Grade II, $A4,400 to $A6,000.

Salaries of the A scale are equivalent to University rates and will be subject to future movements of University salaries.

Appointments will be made within the above ranges according to qualifications and experience.

Application forms and further details available from Queensland Government Offices, 392/393 Strand, London, W.C.2.

Closing date—June 15, 1970. (2086)

BIOCHEMIST

The Centre needs a biochemist to develop methods of labelling with carbon-14 and tritium using enzymatic or microbiological synthesis. The work requires a continuing awareness of the needs of users of these products in university and medical research units in this country and abroad.

Applicants should possess a good honours degree and have at least two years' post-graduate experience in research and development. The starting salary, determined by the successful candidate's age and experience, will be within the range £1,630–£2,895.

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Application forms may be had from the Personnel Officer (Ref: S.162)

THE QUEEN'S AWARD TO INDUSTRY 1967 1970

The Radiochemical Centre
Amersham Bucks

(2045)

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(2105)

GEOLOGISTS

COMMONWEALTH OF AUSTRALIA

Bureau of Mineral Resources

These are senior positions offering first class career opportunities. The appointments are based in Canberra, but periods of field duty will be called for from time to time.

Position 1—($A6,868–$A7,573)—to check samples supplied from drilling operations and arrange for palaeontological and petrological examination of cores and cuttings. Examine all cores and report on lithology and sedimentary structures in a form suitable for publication. Prepare critical comments, for publication, on final reports. Visit drilling operations to check on methods and equipment used by well-site geologists.

Position 2—($A5,905–$A6,631)—To undertake complex studies (in both field and laboratory) of sediments for determination of their sources, environment of deposition and post-depositional history. Prepare scientific reports, plans, sections and diagrams dealing with these investigations. Supervise subordinate staff as required.

Qualifications—Appropriate science degree at good honours level or higher degree together with considerable related experience. For position 2, experience in carbonate sedimentology will be preferred.

Salaries—Commencing salary for both positions will depend on qualifications and experience ($A1 = 9/4d. sterling).

Allowances—Travelling, camping, district and overtime allowances are payable where applicable.

Conditions—First class passages, by sea or air for successful applicants and their dependants, together with generous baggage allowances. Salary paid from date of embarkation. Three weeks' annual leave and cumulative sick leave provisions. For British subjects there is a comprehensive Superannuation Scheme.

Application forms may be obained from:

The Recruitment Officer,
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Canberra House, 10–16 Maltravers Street,
Strand, London, W.C.2

Please state in which position you are interested.

(2064)

University of Leiden, The Netherlands

Faculty of Medicine

CHAIR OF PHYSIOLOGY

Applications are invited for an appointment to the above-mentioned chair, rendered vacant by the death of Professor J. W. Duyff, M.D.

The appointment will carry with it the responsibility for co-ordinating physiological research projects within the Department of Physiology as well as in clinical departments of the University Medical Centre, and preference will be given to applicants with research interests related to clinical physiology.

Further particulars may be obtained from, and applications should be submitted to, the Advisory Committee,

c/o Professor E. L. Noach,
Department of Pharmacology,
Wassenaarseweg 62,
Leiden.

(2103)

UNIVERSITY OF GLASGOW

LECTURESHIP IN PHYSIOLOGY

Applications are invited for a LECTURESHIP IN PHYSIOLOGY. Salary within the range £1,240 to £2,850 per annum on the Lecturers' scale (presently under review). Initial salary will be fixed according to experience and qualifications. F.S.S.U.

Applications (eight copies) should be lodged not later than June 4, 1970, with the undersigned, from whom further particulars may be obtained. In reply please quote Ref. No. 3026-M.

ROBT. T. HUTCHESON,
Secretary of the University Court.

(1992)

MACQUARIE UNIVERSITY SYDNEY, AUSTRALIA

Applications are invited for appointment to positions in the fields indicated below. The positions are tenable from the commencement of 1971. Normally appointees should take up duty by mid-February, 1971. Later dates of entry on duty may be arranged in special cases.

SENIOR LECTURERS/ LECTURERS

BIOLOGY

School of Biological Sciences (lecturers only, in the fields of animal physiology, ecology and animal population or biochemical genetics).

CHEMISTRY

School of Chemistry (lecturer only, any field).

The salary for a Senior Lecturer will be within the range $A7,600 by $A230 to $A8,750 per annum; and for a Lecturer $A5,400 (6) by $A270, (1) by $A280 to $A7,300 per annum. These salaries are at present under review.

Macquarie University occupies an attractive site in Sydney, and is planned to develop rapidly over the next decade. The University commenced postgraduate teaching in 1966 and undergraduate teaching in 1967. Some 4,200 students are currently enrolled including approximately 400 postgraduate students. The organisation of the University provides considerable flexibility in the construction of degree courses for individual students and provides for interdisciplinary studies.

Further information, including advice regarding the method of application, should be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, Macquarie University, North Ryde, New South Wales 2113, Australia.

Applications close on June 26, 1970.

(2068)

UNIVERSITY OF SOUTHAMPTON

INSTITUTE OF SOUND AND VIBRATION RESEARCH

Applications are invited for the post of Shell-Mex and B.P. Lecturer. There will be lecturing commitments in human factors at both undergraduate and postgraduate level. In addition to primary interests in sound and vibration, the successful candidate will be expected to conduct and organize research in a wider variety of human factors, with particular reference to the transportation environment. Salary scale £1,730 to £3,105, with a merit bar at £2,230. F.S.S.U.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton, SO9 5NH, to whom applications should be sent before May 30, 1970, quoting ref.: N

(2097)

NATURE

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(Continued overleaf)

Chemical Applications of Far Infrared Spectroscopy

A. Finch, P. N. Gates, K. Radcliffe *Royal Holloway College, Surrey, England*
F. N. Dickson *Mellon Institute, Pittsburgh, Pennsylvania, USA*
and F. F. Bentley *Materials Division, Wright-Patterson Air Force Base, Dayton, Ohio, USA*
May 1970, viii+278 pp., 100s. (£5.00)

This book is an account of the applications, techniques and instrumentation of far-infrared spectroscopy, and interferometry: a definitive work intended for students, researchers and industrial workers concerned with the application of vibrational spectroscopy to problems of chemical interest.

Contents Introduction. Instrumental considerations in the design of far-infrared spectrometers. Techniques. Barriers to internal rotation: Torsions. Hydrogen-bonded and charge-transfer systems. Complex inorganic systems. Metal-organic and organic compounds. Spectroscopy of crystal lattices. Author index. Subject index.

Space through the Ages

The evolution of geometrical ideas from Pythagoras to Hilbert and Einstein
C. Lanczos *Dublin Institute for Advanced Studies, School of Theoretical Physics, Dublin, Ireland*
May 1970, x+320 pp., 80s. (£4.00)

This book presents the philosophical logic of deduction and induction in the development of the aesthetic concepts of man's perception of space. As a history of geometrical thinking, this work will interest all mathematicians, logicians, philosophers, and physicists, as well as students who have an understanding of the basic concepts of algebra and calculus.

Contents Early beginnings. Greek geometry. The evolution of metrical geometry. Tensor algebra. Tensor analysis. The geometry of Gauss and Riemann. Einstein's theory of gravitation. Abstract spaces. Projective geometry. Bibliographical index. General index.

Advances in Microbial Physiology Volume 4

Edited by A. H. Rose *School of Biological Sciences, University of Bath, England*
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This series presents critical review articles on all aspects of the physiological and biochemical activities of micro-organisms. It deals with subjects of interest to microbiologists and biochemists which have until now received little attention from reviewers.

Contents Biosynthesis of secondary metabolites: roles of trace metals. Antimicrobial agents and membrane function. Encystment in amoebae. Serotype expression in paramecium. The aliphatic amidases of *Pseudonomas aeruginosa*. The place of continuous culture in microbiological research. Catabolite repression and other control mechanisms in carbohydrate utilization. Author index. Subject index.

Methods in Microbiology Volume 3A

Edited by J. R. Norris *Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent, England*
and D. W. Ribbons
Department of Biochemistry, University of Miami School of Medicine, and Howard Hughes Medical Institute, Miami, Florida, USA
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Contents Media for the maintenance and preservation of bacteria. Culture collections and the preservation of bacteria. Design and formulation of microbial culture media. Quantitative relationships between growth media constituents and cellular yields and composition. Enrichment cultures of prokaryotic organisms. The isolation of mutants. Improvement of micro-organisms by mutation, hybridization and selection. Author index. Subject index.

FEBS Proceedings of the Sixth Meeting, Madrid April 1969

Volume 19: Metabolic Regulation and Enzyme Action

Edited by A. Sols *Institute of Enzymology, Madrid, Spain*
and S. Grisolia *University of Kansas Medical Center, Kansas City, Kansas, USA*
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It presents a very significant proportion of the current research trends in key areas of these two fields, particularly in such fundamental problems as gene expression, complex metabolic pathways, interconvertible enyzmes, active sites, modulation of enzyme activity, and molecular bases of inborn metabolic errors.

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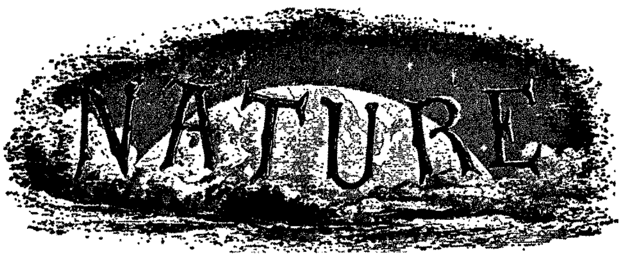
Knowing Where to Draw the Line

THE decision to hive off the special laboratories at MIT is at once sensible and courageous. The Lincoln Laboratory and the Draper Laboratory have been thorns in the institute's flesh for at least two years. More than a year ago, in March 1969, they were the focus of the one-day strike against too close a mixture of military and academic research which spread outwards from MIT to several other universities. Before and since, the special laboratories at MIT have also become familiar parts of the protests of the students against the establishment. But openly to acknowledge that the step now to be taken to separate the two laboratories from the institute is a step in the direction which the students have been urging is not a victory for out and out radicalism—indeed, for the more extreme among the students, the steps now taken may not go far enough (see page 793). The truth is that the anomalies which the Lincoln and Draper Laboratories have created go back much earlier than the past few years of campus discontent. For at least a decade, entirely level headed people have been saying that MIT had become too dependent on the overheads to be earned from military research. The fact that the institute will now have to look elsewhere for $8 million a year, roughly one seventh of its present budget, at a time when all universities are feeling the pinch is a proof at once of the degree to which MIT has come to rely on subsidies of this kind and of the courage—possibly the courage of despair—with which Dr Howard Johnson has faced up to the need for a clear decision.

The origins of the defence laboratories associated with universities are to be found in the years of hectic preparation for military defence during the Second World War. The Lincoln Laboratory is itself the descendant of the old Radiation Laboratory, at which much American work on radar and associated devices was carried out. Then and since, several American universities have found themselves committed to the management of laboratories like these. Thus the University of California has been ostensibly the manager of the weapons laboratories at Los Alamos and Livermore since the end of the Second World War. In another field, possibly a less contentious one, the California Institute of Technology manages the Jet Propulsion Laboratory on behalf of NASA. Then there are laboratories like the research institutes at places such as Stanford (and until recently at Buffalo) where the natural interests of previous faculty members have led to the setting up of specialized laboratories which live from year to year on military contracts. In the past quarter of a century, these laboratories have performed a valuable service for the military and, by extension, for the United States as a whole. In some cases, but not all (Los Alamos is an obvious exception), the intimate connexion between the university and the laboratory has been of benefit to the latter and sometimes even to the former. In other cases, the mutual benefits of these arrangements have been more mundane in character—defence laboratories managed by universities rid the United States Government of direct responsibility for the continuity of a research programme, and also provide a convenient means by which the salary scales of the public service do not have to be strictly applied. For their trouble, the universities are provided with a frequently handsome overhead. In the present climate, it is hard to know which of these arrangements is the more liable to attack. The argument that the universities should never be associated with military work is obviously untenable—if academics paid no attention to military research, they could even be held to be neglectful of making a proper contribution to the unfortunately necessary task of national defence. Moreover, in the past few years, it has become apparent that much of the research that goes on in university departments has some commercial value, which means that the old simple doctrine of complete access to all academic information has had to be shaded to a significant extent. There is, however, a clear difference between the part-time involvement of a part of an academic's energy in some secret project and the employment by universities of large numbers of full timers all busily engaged on secret projects. The former may be acceptable, but the second has several obvious drawbacks, not least of which is that it can seriously debase the currency of academic life. This is one argument against some of the special laboratories where the university connexion has been strong and intimate. At the other end of the scale, as with Los Alamos, for example, the connexion has been so tenuous that the University of California has always been able with justice to assert that the laboratory has had no adverse effect on the quality of academic life, but this, of course, has laid it open to the charge that it has been living not merely off illgotten but off unearned gains. And tactically, of course, the universities which depend on the overhead contribution from research projects which are not directly under university control are likely to come a cropper any day if there should be a sudden cut in the budget of some mission oriented agency. Dr Johnson's administrators at MIT may find in the weeks ahead some wry comfort in the thought that if they had not voluntarily forgone their commission for operating the special laboratories, the money may have been snatched from them next year by some act of Congress or of the Administration.

Several questions now arise, not the least of which is the likelihood that other universities will find themselves following MIT (California Institute of Technology may yet be lucky that its student body remains one of the most docile, although there is also no doubt that the Jet Propulsion Laboratory is a somewhat different kettle of fish from the Draper Laboratory at MIT). The issue is already alive in the University of California and the chances are that the continued management of Los Alamos and Livermore will be impossible. It is therefore only fair to acknowledge first of all that useful laboratories should have room to carry on if their cosy but anachronistic relationships with universities are severed. It is also important, but in the present climate less likely to be acknowledged, that universities should be helped with money to carry out the research likely to be denied to them now that the overhead contributions with which they have financed a good deal of their internal research are likely to be snatched away.

100 Years Ago



PERHAPS the most extraordinary instance of excessive and depraved appetite on record is that of a French soldier, named Tarare, whose case is described in vol. XXI. of the *Dictionnaire des Sciences Médicales*, by Dr. Percy. He was born near Lyons, and came up to Paris, where his first exploit was to eat a basket of apples—at a friend's expense. On various occasions he swallowed a series of corks and other indigestible materials, which produced such violent colic that he was obliged to attend the Hotel Dieu, and whilst being examined almost managed to swallow the watch-chain and seals of the surgeon in attendance, M. Giraud. Desault, on the occasion of one of these attacks of colic, tried to frighten him out of his gross habits by declaring that it would be necessary to open his stomach, and arranged the instruments; he ran away, and relieved himself by copious draughts of warm water. Soon after he found that his appetite had really increased to an excessive amount, probably owing to the continued irritation produced by these absurd tricks.

In the presence of some officers he swallowed, at one sitting, 30lb. of liver and lights. His insatiable appetite was for once in his life made useful, by his being selected to convey a correspondence between General Beauharnais and a French colonel, which was inserted in a box and swallowed; but he was caught and soundly thrashed. He fell under suspicion of having eaten a child fourteen months old. It is stated that he was of mild and gentle manners and aspect. After death his stomach was found in a very diseased condition.

From Nature, **2**, *90, June 2, 1870.*

OLD WORLD

INCOMES

Threat to FSSU Pensions

AN unpleasant and entirely unnecessary surprise is to face 35,000 university teachers in the Finance Bill, which is scheduled to be debated in the House of Commons this week. As members of the pension scheme operated by the Federated Superannuation System for Universities (FSSU), they have enjoyed particular flexibility in the way they receive their retirement benefits, as well as particularly generous provision for dependants in the event of death during service. Unless the relevant clauses are excised from the Finance Bill this week, the value of the FSSU scheme may be severely eroded.

What seems to have happened is that people with larger incomes than FSSU members have exploited the rules of this type of pension scheme as a means of evading tax. The Department of Inland Revenue has worded the new Finance Bill so as to close this loophole, but whether through incompetence, oversight or plain indifference has apparently ignored the fact that FSSU members will be caught in the net as well as the tax evaders.

The basis of the FSSU scheme is that a member contributes 5 per cent of his salary each year towards the scheme and his employer contributes a sum equal to 10 per cent of salary. The pension, which in fact is more like a life assurance, can be received in the form of a lump sum on retirement.

At present the employer's contribution is not considered part of the member's taxable income and in at least two previous finance bills special exceptions have been made to ensure that FSSU contributions remain untaxed. The new Finance Bill makes no exception for the FSSU. Moreover, it is clear that the FSSU will not be counted by the Inland Revenue among the approved list of pension schemes that are exempt from tax.

This means either that the UGC, as paymaster for the universities, will have to make up the added burden of taxation on university teachers' salaries, which it is not prepared to do, or that the FSSU must conform to the new regulations for approved schemes. These, in effect, would destroy the flexibility of the FSSU by restricting the pension payable in a lump sum to a small fraction of the total amount due, the rest being received in weekly payments.

FSSU members and those who represent them may be placing too much hope in a clause of the Finance Bill which gives the Inland Revenue discretion to waive the tax on employers' contributions. Despite the discussions held with the Association of University Teachers (AUT) and the council of the FSSU, the department has apparently given no sign of being prepared to let the FSSU slip through its indiscriminate net.

Perhaps the worst aspect of the Finance Bill in its present form is that if it becomes law it will be retrospective in its application; present members of the FSSU will be denied the kind of benefits to which they had always believed their contributions would entitle them.

TRANSPORT

All Stations to Heathrow

THE best way of carrying passengers from Heathrow airport to the centre of London would be to extend the Piccadilly underground line from Hounslow West to the airport, according to a study group set up by the Ministry of Transport. The extension, which London Transport would be able to build by the mid 1970s, would be 3·5 miles long and might carry 17 million passengers a year by 1981. Alternative schemes for a British Rail link with Heathrow should be ruled out because they would cost up to twice as much as the estimated £19 million for the underground link, the study group concludes in its report (*Rail Links with Heathrow Airport*, Part I; HMSO, London, 12½p).

If, as London Transport claims, the Piccadilly line will be capable of absorbing the extra traffic, this choice will have several further advantages. Passengers would be dispersed easily into the underground network at interchange stations, whereas the proposals by British Rail for a ground level railway would involve bringing everybody to Victoria station and leaving them to find their own means of transport for the onward journey. Workers at Heathrow could also use the underground line because a station could be built in the airport maintenance area, and fewer existing buildings would need to be demolished if the line were to go under the ground.

The London Transport scheme is favoured by British European Airways and the British Overseas Airways Corporation, but the British Airports Authority, which runs Heathrow, would prefer a British Rail link because of its belief that conditions on the underground line would be too uncomfortable. In the early morning overcrowding might be serious, because arrivals of transatlantic flights would clash with the heavy commuter traffic which makes tube travel unpleasant enough as things are. Getting baggage out of the underground trains at each station might lead to cumulative delays, and in any event the journey would be quicker by the non-stop services that British Rail has proposed.

SCHOOLS

Wooing Children to Science

by our Education Correspondent

ENCOURAGING more schoolchildren to opt for mathematics and for science subjects in the sixth form is a long-standing problem which has no easy solution. But this week the Joint Mathematical Council, the Royal Institute of Chemistry and the Institution of Chemical Engineers have published documents which might help to relieve the situation. On the one hand, the Joint Mathematical Council is concerned that mathematics teaching should be kept up to date and, on the other, the Royal Institute of Chemistry and the Institution of Chemical Engineers are concerned to improve the image of careers in chemistry.

The Joint Mathematical Council has published a statement on the present state of change in mathematics at secondary school level, in which it sets out the principles which underlie what it considers to be the best features of the new mathematics curricula. The aim of the statement is to provide a basis for discussion of so-called "modern mathematics" teaching. One problem is to decide what aspects of mathematics to teach, and to relate school curricula to recent advances in mathematics. But perhaps the most pressing problem is how to keep mathematics teachers fully informed of developments in teaching methods. The most effective way, the statement says, is to provide more facilities for in-service training.

The council believes that centres should be established in several parts of Britain, to provide in-service training on a regional basis. Such a centre is already operating in Nottingham, where staff from the University of Nottingham and from local colleges of education go out to schools to explain the thinking behind new courses. This approach, the council believes, is more effective than providing standardized courses for serving teachers.

Looming large over the council's thinking are the proposals for changing sixth form curricula and examinations. The council particularly wants to be sure that the proposals will not hold back the mathematically gifted child, and that they will not aggravate the shortage of mathematics teachers. It has set up a working party to consider the implications of the proposals for mathematics teaching, and the council will consider a report from the working party when it meets next week.

Improving the standard of teaching might help to attract students to mathematics, but perhaps the most effective incentive is the prospect of an interesting and highly paid career. It is with this thought in mind that the Royal Institute of Chemistry and the Institution of Chemical Engineers have launched a pamphlet which tells of the advantages to be gained from a career in the chemical industry. The pamphlet is aimed at children aged between thirteen and fifteen who, under the present system of specialized courses, will be taking crucial decisions about their future education. The Royal Institute and the Institution of Chemical Engineers believe that the need to convert more people to chemistry is so pressing that there is no time to wait for changes in sixth form curricula to take effect.

MEETINGS

Astronomers Meet at Brighton

THE Regency exuberances of Brighton will be echoing to talk of quasars and pulsars this summer. For the first time since 1925, the triennial general assembly of the International Astronomical Union is meeting in Britain, at Brighton and on the nearby campus of the University of Sussex. On the first and last days of the meeting, August 18 and 27, the administrative affairs of the union will be conducted, but the main business will be the meetings of the forty or so commissions into which astronomy has been successfully compartmentalized by the union. There should be plenty to talk about.

Commission 15, for example, is concerned with the physics of comets, and the members will be keen to discuss what to make of the vast hydrogen clouds which surround comets Tago-Sato-Kosaka and Bennett. Then there is commission 17, charged with lunar affairs, which has its hands full with trying to perfect a system for naming lunar features. At the last general assembly at Prague in 1967, hundreds of names were proposed for features on the far side.

As an antidote to specialization there will be invited discourses by A. Hewish and V. L. Ginzburg on pulsars, and by B. J. Bok and C. C. Lin on the spiral structure of galaxies, and a special meeting on the interpretation of results from Apollos 11 and 12.

About fifty countries support the IAU financially, but the union is almost unique in that the membership is made up of individuals who do not pay dues. More than half of the present membership of 2,000 are expected to visit Brighton for this year's general assembly.

MONGOLIA

Tidings from Ulan Bator

THE president of the Mongolian Academy of Sciences was in Britain last week to receive an honorary degree from the University of Leeds. The Snow line is not recognized in Mongolia, and the academy over which Professor B. Shirendev presides includes institutes of literature and history as well as of science. The natural sciences are studied in five institutes devoted to physics and mathematics, chemistry, biology and botany, geography and geology, and the academy also supports an observatory.

Modern science has only been studied in Mongolia since the revolution in 1921, but sciences such as astronomy have a venerable tradition, which was kept alive in the monasteries and other seats of learning. Besides the institutes maintained by the academy, the chief places of scientific study in Mongolia are the universities and the research institutes attached to particular ministries. Altogether the scientific strength of the country numbers about 2,000 research workers.

The budget for the Academy of Sciences is about 30 million roubles or £3 million. Professor Shirendev was unable to specify the total expenditure devoted to scientific research in Mongolia, but in any event his government is amply recompensed by the fruits of the research it supports. The Academy of Sciences is proud of its close links with scientists in other countries and that it numbers foreign scientists, such as Professor Owen Lattimore of Leeds University, among its members.

NUCLEAR PHYSICS

Prospects at CERN

AN important decision that is likely to confront the Secretary for Education and Science in the early months of the new parliament is whether or not Britain should back the revised version of the CERN 300 GeV accelerator project. Renewed pressure on the government is already mounting. As the first shot in the campaign, a group of leading British physicists signed a letter to *The Times* last week which urged the government to give the new proposals strong support now that the British balance of payments position has regained its health. The Science Research Council also disclosed this week that it has been considering the new proposals informally and greatly welcomes them. It is now awaiting detailed design studies for the new accelerator.

In the original 300 GeV scheme the goal was a clearly defined energy for which all parameters in the design were optimized. The cost was therefore well known in advance. In what may be called the creeping GeV plan put forward recently by Dr Adams, director of the 300 GeV project, the maximum energy can be increased in a series of stages. This means that at relatively little extra cost per GeV there is a guarantee of some value for money at each stage of expansion. Thus the initial expenditure for achieving energies up to 150 GeV would be about two thirds that for the original 300 GeV plan, but the incentive to go to higher energies would be very much stronger. The new scheme could turn out to be not only a way of making high energy physics more palatable to governments but also a means of ensuring the continued expansion of the subject if the research looks promising. Certainly if the development of superconducting magnets proceeds as well as is hoped, the demand for channelling more funds into high energy physics would become very strong.

There are, of course, other considerations which make the new proposals attractive. CERN has long been held as an example of how international collaboration can work, and the revised plan would remove the previous stumbling block of where the accelerator should be housed, as there would be room for it at the present site near Geneva. Moreover, the British Government would have to find a well chosen argument to fend off the claims of CERN for the second time in three years.

CONSERVATION

Burgeoning of Beavers

from our Soviet Correspondent

THE threatened beaver population of the Ukraine is now making a comeback. With the help of conservation measures and infusion of fresh stock the beavers have extended their range to the coasts of the Black Sea, according to a report from the Kiev Institute of Zoology.

The Ukrainian beaver was hunted extensively as a source of meat and fur throughout the historical epoch, from the time of the grand princes of Kiev (9th–13th centuries) until 1927, when the maximum beaver population of the Ukraine was estimated as being less than 80 individuals. Strict conservation laws were at once introduced and in 1934–35 some restocking was carried out. Canadian beavers were

introduced on the headwaters of the Horyn. Unfortunately, the beaver population of Ukraine was once again almost wiped out, this time by the military operations of the Second World War. The Zhitomir and Kiev regions were restocked with some 50 animals from Byelorussia in the early post-war years and by 1965 the total beaver population of Ukraine was estimated at 2,000, situated, according to the *Ukrainian Soviet Encyclopedia* (vol. 17, 1965) on the Dnieper, Teperev, Pripyat, Desna and Horyn rivers, the greatest concentration being on the areas newly flooded in connexion with the construction of the Dnieper dams at Kiev and Kaniv. Further expansion of the beavers' range is being assisted by careful trapping and subsequent release of suitable specimens (numbering some 400 to date) into rivers with no surviving indigenous population. This resettlement, together with the natural expansion of the population (in a general southward and eastward direction), gives the beavers a more promising future than they have had for many years.

EXAMINATIONS

More Evidence against A-Levels

UNIVERSITIES should not select their students on the basis of 'A' level results alone, and other criteria, such as a student's outside interests, his headmaster's report, and his attitude to his future career, should be given much more weight in the selection process than they are at present. These conclusions, drawn by P. R. Freeman of University College, London, are based on an analysis of the performance of students in first year examinations at the University of Reading (*Journal of the Royal Statistical Society*, **133**, 38; 1970).

The variables used in the analysis were the results of questionnaires designed to measure students' intelligence, motivation and personality, and also taken into account was an autobiographical sketch in which the students were asked to imagine they had just reached the age of sixty. There were also seven scholastic variables including 'A' level results, 'S' level results and headmaster's report. 'A' level results turned out to be the best single indicator of university performance, but the headmaster's report, the student's projective autobiography and some personality factors also proved to be valuable guides to his academic ability as measured by university examinations. But admissions tutors should be wary of using intelligence tests and personal interviews as a basis for selection because, according to Dr Freeman's analysis, none of the chief components of a student's psychological "make up" are strongly associated with his academic ability, and intelligence tests are not a very useful guide.

These findings, which broadly agree with those made by D. G. Bagg from a study of the performance of engineering students at Manchester (*Nature*, **225**, 1105; 1970), should be seen as strong grounds for eschewing the emphasis on 'A' level performance for university selection, Dr Freeman suggests. Such a change in emphasis, and its effect on sixth form syllabuses, would be most welcome to the advocates of broader sixth form curricula, and improved ability to predict a student's performance could also be used to provide an early warning if his standards start to fall.

Dr Freeman is careful to point out that there are many possible variables not included in the analysis. For example, the content of degree courses and the quality of the teaching would be expected to influence a student's performance and there is, in any case, no guarantee that university examinations are the best method for assessing a student's ability.

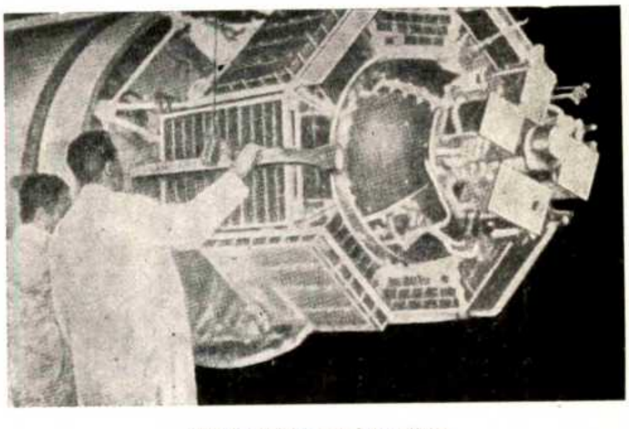
SATELLITES

Polarized Bursts

from our Soviet Correspondent

AN interesting feature of the conference now being held by the Committee on Space Research (COSPAR) in Leningrad is the publication of the preliminary results of the experiments performed by the first of the Interkosmos series of satellites (see *Nature*, **225**, 117; 1970). Interkosmos–1, launched on October 14, 1969, carried instrument packages provided by Czechoslovakia, East Germany and the Soviet Union, designed to measure the ultraviolet and X-ray radiation of the Sun and its effect on the upper atmosphere. The satellite had a perigee of 240 km and an apogee of 640 km.

Among the preliminary results, observations of solar bursts between October 20 and 30, 1969, indicate that the X-ray emission from solar bursts is partially polarized, the coefficient of polarization varying as the burst develops, which is evidence that this radiation is not purely thermal in origin. A correlation between the X-radiation from bursts and processes in the lower ionospheric has also been observed, which, of course, is known to have considerable significance for long-wave broadcasting.



The Interkosmos-I satellite.

Other results include data on the composition of the upper atmosphere (obtained by absorption measurements with a Lyman-alpha photometer) and optical photometer determinations of the density of aerosols produced in the upper atmosphere by meteoric dust. (The exact figures for these experiments are yet to be published.)

Interkosmos–1 had an orbit very close to those of the Earth satellites Kosmos–166 and 230 which observed the Sun. It is expected that when the complete data of Interkosmos–1 are published, they will include not only the results of the Russian, Czech and East German experiments but a comparison with the data obtained by its two Kosmos predecessors.

UNITS

Going Metric in Britain

THE Metrication Board published its first report earlier this month (*Going Metric—The First Five Years*. HMSO, 12*s* 6*d*). Professor M. L. McGlashan, a member of the board, writes as follows:

The changeover from imperial to metric weights and measures throughout the whole of Britain's industry, commerce, and everyday life had been set in motion by the government in 1965, with 1975 as the target date for its substantial completion. The Metrication Board was appointed just a year ago to catalyse the changeover. When the Board began its work much had already been achieved: by the British Standards Institution, especially in the preparation of metrication programmes for various sectors of industry, by government departments, by the professional and trade associations, by the Industrial Training Boards, by educational associations of all kinds, and by many other institutions, firms, and individuals. Metrication was on its way; the point of no return had already been reached. It remained to be seen only how efficiently and quickly it could be carried through; for, by and large, the earlier and more thoroughly it has been planned and the more quickly it is then done, the lower will be the cost. The Board's task was first to inform itself and then to inform others; to help and encourage the leaders and to prod the laggards; and to identify stumbling-blocks and see that they were cleared from the path.

Typically the Board would find that metrication was going well in all except one of a group of similar industries. In that industry the stumbling-block was (say) metric "bobbles". The Board would be told by the firms consuming bobbles that they were anxious to go metric but could not obtain metric bobbles from their stockists. The Board would be told by the stockists that they were unable to obtain metric bobbles from the manufacturers, and that in any case there was no demand for them from their customers. The Board would be told by the manufacturers that they were ready and willing to supply metric bobbles but that there was no demand for them from the stockists. It is the Board's job to solve such puzzles.

One of the early tasks of the Board was to resolve some confusion about just which units were implied by the word "metrication". This task was greatly helped by a statement by the Minister of Technology in the House of Commons on July 24, 1969, that the International System of Units (SI) was accepted by the government as the basis for the changeover to the metric system. In particular the Board has been at pains to correct a view which had unfortunately gained ground that the centimetre should no longer be used, and even that it was not a unit of the International System, or at best a second-class one or a kind of baby-talk. This view arose from the internationally agreed recommendation of a preferred selection of multiples and sub-multiples of the SI units, excluding the centimetre, for use in industry by technicians, for example, in drawing-offices and on the workshop floor. That recommendation had never been intended to apply to science, or to trade and commerce, or to everyday life; and certainly not to education—except in the most specialized technical training.

The Board's hope that no child starting school after the autumn of 1969 would ever again be exposed to the imperial system as his or her "first language" of measurement has largely been realized. In other spheres of education a good start has also been made. Most public examinations will be fully metric (SI) by the summer of 1972 and the rest very soon afterwards. The educational publishers and manufacturers of educational equipment are responding nobly to the demands made on them by the teachers.

In all other sectors of the economy and national life metrication is either being rapidly implemented, as in the construction and engineering industries, or has been realistically and fully programmed; for example, the statutory speed limits on roads will be changed to round numbers of km/h in 1973.

The Board now faces the rapidly expanding task not only of catalysing all these changes but also of informing the public in good time about them, a task which will reach its peak as, beginning in 1972 and accelerating in 1973, metrication begins to appear in the market place: in the measures used for the sale of petrol and coal and gas and of butter and milk and beer.

The Board does not pretend that metrication can be achieved without cost, but emphasizes that no meaningful estimate has been, or can be, made of the total cost; or, for that matter, of the financial gains to be expected, not only from metrication itself in terms of trade with the rest of the world, but also from the standardization and variety-reduction with which it will so often go hand-in-hand. Case histories of a few individual firms which have completed the change suggest that the cost of the necessary investment is a good deal less than had been feared and that the return is quick and considerable.

Ten Universities Too Few

by our Education Correspondent

THE number of places which existing universities can reasonably expect to provide by 1980 is about 50,000 short of what the government wants. This warning, contained in a statement published last week by the Committee of Vice Chancellors and Principals, is based on estimates sent in by individual institutions of how far they could expand if the necessary resources were made available. It was also accompanied by the message that all but one of the government's suggestions for reducing costs are unacceptable to the universities. This will be cold comfort to the Department of Education and Science, which has been trying to find ways of accommodating the demand for higher education as cheaply as possible.

The universities' estimates are based on the particular circumstances of individual institutions and also, it seems, on the assumption that there will be no major changes in the length of courses or in the relations between the universities and other institutions of higher education. The universities reckon that about 400,000 full-time student places is about the most that they can hope to provide by the end of this decade. The Department of Education and Science, on the other hand, has worked out that if 53 per cent of the school leavers with two or more A-levels are going to get into university in 1980, about 450,000 places are required. If the extra places are not found, universities will be forced to put up their entry requirements, and to turn away more and more good students. Opportunities are, in fact, already being reduced because universities are now taking only 49 per cent of qualified school leavers compared with 53 per cent in 1963.

What, then, is to be done? The shortfall could, of course, be taken up by establishing some new universities, but even if this were done almost immediately it would require at least ten new institutions to provide the required capacity by 1980, and Mr Short has already hinted in a written reply that the government has no definite intention of following such a course. The answer therefore seems to lie in some restructuring of the whole higher education system, and the vice chancellors have cast an eye on the colleges of education. But they have been careful not to be too presumptuous because sweeping changes in the system require long study, and in any event it is not their place to make recommendations about other institutions. At the centre of their thinking is the possibility of introducing two-year courses of study to a sub-degree level. This is, in fact, the only suggestion for reducing costs which has been canvassed by the government and which the universities believe requires further study. But they see it as more of an educational measure than a short-term way of saving money.

Such courses could take one of several forms. One suggestion is that they could be given in modified colleges of education to students who could be expected to reach the limit of their academic ability after two years of study. On the other hand, some universities believe that there might be scope for reviewing the university course structure in a way which would permit a basic two year course to be followed by one or two years of study for those wishing and able to proceed to a specialist honours degree or to a higher degree. The two year course could, in this case, be given in a modified college of education associated with a particular university.

Such two year courses are not a new idea. They were suggested by Professor Brian Pippard in the Swann Report in 1968 and again this month in an article in the *Cambridge Reporter*, and there seems to be an influential lobby developing in the vice chancellors' committee for changing the role of the colleges of education. Dr F. S. Dainton, vice chancellor of the University of Nottingham, for example, has suggested that colleges of education should be turned into liberal arts colleges, providing courses to a first degree level from which students could proceed to more specialist courses at the universities (see *Nature*, **226**, 490; 1970). Such a development would, of course, require stronger links to be forged between existing colleges of education and the universities.

A more intractable problem is how the polytechnics are going to develop during the next decade. The government has staked many of its hopes in the polytechnics, and has provided for a rapid expansion in their capacity during the 1970s. But their role has not been clearly worked out. The vice chancellors statement drew attention to the possibility that there will be wasteful duplication of resources if they develop along the same lines as the universities, but, on the other hand, the universities are chary of suggesting that there should be a unified system of finance and control of all higher education institutions. Such proposals, they argue, require much deeper study. But there seems to be general agreement that stronger links must be forged between the polytechnics and the universities and, in Dr Dainton's plan, some polytechnics would be directly affiliated with the universities while others would develop along similar lines to the colleges of education.

As far as cost saving is concerned, the universities have rejected the government's suggestion that they could be more restrictive in admitting students from overseas, that grant-aided students might be required to enter specific types of employment, and that a loans system might replace the present system of maintenance grants for students. All these proposals, they argue, would be socially unacceptable and, in any case, they would not save very much money. The suggestion that some universities should take the bulk of their students from surrounding areas is also unacceptable because students living on the fringes of the "catchment area" would have to spend too much time travelling, and because living away from home is, in many cases, a good maturing influence. The vice chancellors' statement also pointed out that capital grants for residential accommodation have fallen sharply over the last few years, and much greater savings might arise from loan-financed student housing schemes.

One suggestion that the universities are particularly anxious about is that student numbers might be allowed to increase at a greater rate than the number of staff. Because staff salaries form the largest portion of recurrent costs in the universities, such a measure would result in considerable savings. The vice chancellors' answer is that, although there may be a few departments where some change could be made in the staff/student ratio, they would be very reluctant to see it altered to any great extent. For one thing, close personal contact between staff and students, which is only possible if the staff/student ratio is small, is a particular hallmark of British universities, and it is essential for maintaining the standards of university degrees. In any case, the vice chancellors argue that the growth in numbers of full-time staff has not kept pace in the past few years with the growth in student numbers, and the staff/student ratio is therefore already under pressure. And there is little prospect that the universities will have enough money during the remaining years of the present quinquennium to halt this trend.

The government can, however, take a few crumbs of comfort from the universities' obvious intention to do all they can to expand. But the vice chancellors' statement pointed out that a growth rate of some 6 per cent a year would require a government decision to devote a higher proportion of the national product than hitherto to higher education. Such a decision, the statement said, "would be both right in the interests of the nation, and inescapable in view of the prospective demand".

NEW WORLD

No Boom for Atomic Energy

THE Joint Committee on Atomic Energy, the watchdog of the Atomic Energy Commission, has deserved its reputation with the commission's firmest friends by recommending a budget authorization which is \$7·7 million more than the \$2,283 million for which the Administration had asked in February. Without dissent, the budget passed through the Senate and the House of Representatives a week ago, although it remains to be seen whether this larger sum of money will actually be appropriated for the coming year and whether, if it is, the AEC will be allowed to spend it. Those who will have been impressed with the evidence given to the committee about the effect of some of the Administration's policies on the ability of the AEC to support basic research in the physical sciences will no doubt be disappointed that the joint committee has nothing to add to these items in the Administration's budget.

If there is no extra money, however, the committee does have something to say about the consequences of the Atomic Energy Commission's new attitude to basic research. In the year ahead, the commission's expenditure on physical research will be \$274 million, a decrease of \$3·75 million from the preceding year. In addition, the AEC has asked to spend in the coming financial year a total of \$119 million on capital projects, chief among which is the 200 GeV accelerator—now to be made capable of accelerating protons to 500 GeV. The joint committee says that the decision during the coming year to "start to phase out" the proton accelerator operated jointly by Princeton University and the University of Pennsylvania and which has been, according to the joint committee, the mainstay for high energy research for fifteen university groups in the past five years, is most disturbing. The joint committee goes on to say that the shutdown should be postponed at least until the universities can find the money to keep on operating the accelerator, in which light it is more than a little surprising that the joint committee offers no extra money.

In other parts of the physical research programme supported by the AEC, the committee says it is concerned that there is nothing in the 1971 budget for the building of high energy particle storage rooms comparable with those at CERN, saying that the result will be that increasing numbers of American scientists will try to carry out work at facilities abroad, to the detriment of what they undertake in the United States. One of the fields in which the AEC proposes to increase its rate of expenditure is in medium energy physics, chiefly in connexion with the Los Alamos meson physics facility, but the committee takes the view that the plan to build into the machine facilities for biomedical research some time in the future should be paid for with other funds. Without recommending extra money, the committee has also shaken its head over the reduction of expenditure on low energy physics, chiefly by the shutting down of Van de Graaff accelerators at a number of universities. In spite of the enthusiasm of the AEC for thermonuclear research based on variations of the Russian Tokamak devices described in the past year, the joint committee believes that there is still much to be gained from a study of other types of thermonuclear machines and hence it has lopped off \$1 million from the AEC's request.

The joint committee has dealt with other parts of the AEC's programme for 1971 with similar care. One of its more vigorous decisions has been to ask the AEC to spend more effort on the development of liquid metal fast breeder reactors, which is "of the highest priority". The joint committee says that it would like to see more interest in industrially sponsored reactor projects and fears that the amount of money allocated for helping such work along is insufficient. In the coming year, the AEC is planning to spend \$43 million on liquid metal fast breeder reactors, mostly at its own establishments, but something like \$7 million is also being spent on the definition of prototype reactors. The joint committee is doubtful of the significance of this work and has attached to the authorization bill a requirement that the Atomic Energy Commission should give it 45 days in which to consider and approve of proposals for collaboration with industrial companies for the building of experimental reactors. In general, however, the committee does urge that the AEC should build itself a breeder demonstration plant without delay.

The other amendments to the Administration's request for funds recommended by the joint committee spring from its belief that more needs to be done to build up stocks of fissile material for military as well as civil purposes. In particular, the committee quarrels with the AEC's proposal to close one of the two reactors at Richland, Washington, which has been used for the manufacture of plutonium for the best part of two decades; it fears that the remaining military reactors—one at Richland, two at Hanford and three at Savannah River—would not be able to meet a sudden increase in the demand for nuclear explosives that might arise from the antiballistic missile programme now under way. It also asks that the AEC should be allowed to begin work on what is called the Cascade Improvement Program by means of which it has been suggested that the output of enriched uranium from gaseous diffusion plants could be substantially increased. One of the committee's anxieties is that at the present level of operations, it will be hard for the US Government to honour its promise that enrichment services will be available to all qualified customers, in the United States and elsewhere, at a reasonably low cost.

Much of the joint committee's commentary on the AEC's proposals for 1971 is linked with the current preoccupation with the quality of the environment. Several parts of the proposed budget have been increased by modest sums of money. Thus the committee urges the expenditure of an extra \$140 on the food irradiation programme, chiefly so that the commission can carry out more careful studies of the mutagenicity of irradiated foods before applying to the Food and Drug Administration for permission to use them experimentally.

Decision-making on Massachusetts Avenue

from our Cambridge Correspondent

HARVARD and MIT, at opposite ends of Massachusetts Avenue, have had their share of problems in the past year or two. Some have been those of all the thinking campuses of the United States—attitudes to war in South-east Asia, new directions for research into the issues of industrial society and questions as to the political responsibilities of a university. But some have been distinctly their own. Two such which have just surfaced are those of defence related research and whether to support the campaign to make General Motors more socially responsible.

The GM issue has had to be faced by universities and other bodies all over the nation, but the votes of Harvard and MIT (600,000 of 280 million), if cast in favour of Campaign GM's proposals, would have carried more weight than 10 million in most other places. The complex and often incestuous relations between the governing bodies of Harvard and MIT and that of GM have made the decision the more ticklish and the outcome perhaps the more inevitable. Neither voted in favour of the campaign.

This came as a shock to many of the eastern liberals who have been pleasantly surprised to see august institutions gaily distributing academic time to teach-ins, teach-outs, walks, canvasses, rallies and strikes on the Cambodian issue. Indeed, the attention that has been focused on Cambodia has taken quite a lot of the steam out of the apparently more mundane issue of bringing the mammoth corporation to heel, while the general dispersal of students at the end of their term has made it easier for the decision to be taken without bricks being thrown through treasurers' offices. In hindsight, it is clear that students have put too much emphasis on the next to impossible—shifting middle America—instead of the not improbable—embarrassing an unloved giant.

University investment policy has never until now been a wildly exciting issue (the Church of England has more experience of unfortunate financial involvement in the nineteenth century). But universities which like to regard themselves as centres of enlightenment ought also to be spreading this enlightenment into some regions where they have an unusual opportunity to make their voice heard. Of course, Campaign GM was bound to fail first time round, but there should be no stigma for a university in backing a loser. One suspects that although executive committees charged with the job of deciding how to vote were scrupulous in excluding from the decision making anyone with a direct interest in GM, they are bodies too tight knit to break ranks entirely with their colleagues.

The statement issued by MIT's Executive Committee bears examination. It had received strong representations from official channels to support Campaign GM, but eventually decided by an "extremely narrow margin" to vote against the proposal to enlarge the board of the corporation. After conceding that the constitution of boards of large corporations is an appropriate matter for public concern, the committee declared, however, that it could not reach agreement that the proposed addition of three to represent consumer interests would be better than leaving the board at its present size. It agreed, however, that there ought to be a greater diversity on the board than there is at present and gave General Motors the gratuitous advice that it should add new members or inject new blood. The difference between taking this stand and supporting Campaign GM is subtle if it exists at all. Perhaps the argument whether there should be 23 or 26 on the board was just a debating point to enable the committee to get itself out of an embarrassing corner. It knows full well that in company affairs, votes are what count; the best way to hide support for the status quo is to give apparent moral support and praise to the opposition.

An equally thorny issue came up with President Howard Johnson's statement to the MIT faculty on Wednesday, May 20, of his decision about the relationship of the institute to the Special Laboratories. The care with which the problems of Lincoln Laboratory and Draper Laboratory have been considered is remarkable at a time when many universities were happy to rid themselves of defence laboratory commitments at the sight of the first radical on campus.

The issues were basically these. Draper Laboratory (formerly Instrumentation Laboratory), situated in the middle of the MIT campus, has an unrivalled pre-eminence in inertial guidance systems. It has held contracts for Poseidon developments, and much of the MIRV technology springs from the expertise of the laboratory and the singular Dr Charles Stark Draper. At the same time, the Draper Laboratory justifiably gets the credit for the remarkable accuracies of navigation in the Apollo missions. Beyond that, its capabilities are only those of any thoroughly competent industrial contractor. Its staff are distinctly more patriotic than is fashionable these days in Cambridge, Mass.

Lincoln Laboratory lives twelves miles out and has a much broader base. It has no presiding genius, its staff are lukewarm to MIT (one member epitomized it by describing his relations with MIT as hitting a monthly high when he sees the magic words on his pay-check), but it has a much more stable base. Words like data, systems, networks, processing, hardware and software—the magic currency of the present—tend to evoke favourable responses at Lincoln. The Department of Defense has repeatedly used the pool of expertise in Lincoln to initiate new projects, although these have been dominantly defensive rather than offensive.

MIT operates both laboratories and so has a very large pool of talent, unlike that of almost any other educational establishment, under its ultimate authority.

An analogy is bound to be incomplete, but it is as if the University of Reading had the Royal Aircraft Establishment at Farnborough under its wing.

The decision which President Johnson took on himself last year was to come to a conclusion within less than a year on whether such attachments were appropriate. The further restraints were that the Executive Committee had barred work on systems for operational deployment as weapons and that financial support had to be forthcoming for any diversification. Add to this the ingredient that MIT, by acting as host to these basically contract laboratories, receives about $8 million annually for administration overheads at a time when the institute is beginning to go into the red, and it appeared that Dr Johnson had a variety of fairly unpalatable options and no easy way out.

His personal standing among most people is, however, high, almost unique for university presidents, and his unassuming nature has probably helped where rhetoric has failed. Another unusual ability in a nation that lives on press leaks is his skill in keeping a secret. Three days before the meeting "informed sources" had told the *Boston Globe* that MIT would keep the laboratories. When he announced his decision, it was that Draper Laboratory would have to go.

The rationale was simple. The styles of the two laboratories differ, and Draper could not stop work on weapons without carnage to the laboratory and its employees. There is insufficient demand for civilian inertial guidance to guarantee the same level of employment, and dismantling the expertise accumulated for a very specific purpose would perhaps ruin the laboratory. As Johnson saw it, forcing the Draper Laboratory away from its present objectives would not work, so immediate steps are being taken to move the laboratory first to being an entirely separate entity of the institute and then, within a year, to being a separate corporation whose ties with MIT would be no greater than those of the many organizations spun off from MIT and Lincoln in the past.

Lincoln Laboratory, with a greater potential for diversification, stays with MIT and defence related research continues, although the need for the declassification of projects and closer educational ties is emphasized. The development of the relations between the mission oriented laboratory and the institute is going to be an object of great interest in the next few years. Whether the Department of Defense will change its funding levels in any significant way and whether Lincoln can attract substantial money from outside, only time can tell. Inspired guesses about finance are few these days with the American financial situation so worrying.

It is early to gauge yet what response the decision to divest the Draper Laboratory will have from those most vociferous in demanding changes. The most strident demands were that the institute retain control and stop war research and among such people there will be bitterness that in years to come the Draper Laboratory will be indistinguishable from most other members of the military industrial complex. However, little constructive advice has been forthcoming about how Draper's expertise can be used to solve America's domestic problems. It looks very much as if Howard Johnson's decision is the least of the possible evils and is probably as little self-destructive as any decision could be under these circumstances.

AIR POLLUTION

Down with Sulphur Dioxide

A COMMITTEE of the National Research Council, set up last year in response to a request from the Department of Health, Education and Welfare, has now published a proposal for a five-year research programme intended to provide ways of reducing concentrations of sulphur dioxide in the atmosphere. High on the committee's list of suggestions is that there should be more research on methods of removing sulphur dioxide from waste gases, principally the exhausts from central power station boilers. The committee was driven to this view by its discovery that there is at present no "commercially proven technology for controlling sulphur oxides", and it goes on to say that in the present state of technology, it would be unwise to force widespread use of unproven processes. This opinion will be something of a blow for the government agencies at present seeking to encourage the adoption of devices for removing sulphur dioxide from power station exhausts.

The committee argues that if present tendencies were to continue, the amount of sulphur dioxide released into the atmosphere in the United States would be four times as great as at present by the year 2000. The forward projections for electricity production in the United States suggest that the total generating capacity will amount to one million MW by 1990, more than three times as much as at present. Coal and oil will be used on an increasing scale until well after the year 2000, although nuclear generating capacity will be greater than that of coal burning stations before the end of the century. In other words, nuclear energy production will not prevent an increase in sulphur dioxide production from generating stations in this century, especially because the increasing use of low grade coal implies that more sulphur is released. Indeed, by the end of the century, it is calculated that 125·8 million tons of sulphur dioxide will be released to the atmosphere from United States industrial operations, close on 80 per cent of it from power stations.

The committee has evidently spent much of its time wondering whether the best way of controlling sulphur dioxide in the atmosphere would be to accelerate the development of nuclear power stations and particularly the breeder reactors without which rapid development would not be possible. Not surprisingly, it comes to the conclusion that this would be to put the cart before the horse. So what can be expected of the technical devices at present offered as means of controlling sulphur dioxide emission ? Although it is possible to remove sulphur from fuel oil, the committee calculates that reducing the sulphur content from 2·6 per cent to less than 1·6 per cent would entail an increase in the cost of fuel of between 20 and 35 per cent, the equivalent of 0·7 mills (1 mill=1/1,000 of a dollar) per kW hour. The notion that it might be possible to remove sulphur from coal by washing is a greater surprise—the committee estimates that coal cleaning could remove all but 1 per cent of sulphur from a quarter of the most heavily contaminated coal and 40 per cent of the sulphur in the rest of the coal used for power generation could be removed by washing processes costing as little as 25 c per ton of coal. In the past few years, most attention seems to have been given to methods for removing sulphur dioxide from stack gases, and the committee has examined 25 of these being developed in the United States as well as a number of methods being tried out elsewhere in the world. It says that only one of the processes has so far been installed in a large commercial installation, and it concludes that there is an urgent need for commercial demonstration plants, presumably supported with federal money, to test the feasibility of the more promising devices, probably at a cost of several million dollars. Among the committee's favourites are the limestone processes for removing sulphur dioxide—processes along these lines have been tried out in Britain since the 1930s, but it seems to be agreed that their efficiency and effectiveness leave much to be desired.

STUDENTS

Origins of Abuse

from a Special Correspondent

Palo Alto, California

President Nixon's disastrous description of American students as "bums", which has united both university staff and students against him, seems to have sprung from a single incident in his home state, California, and on a single individual's special pleading. The evidence for this is now clear. The fire that prompted the remarks did not even take place at a university and there is no incontrovertible evidence that it was caused by students.

During the momentous last week of April (when the President took the Cambodian decision), he received a letter from an American professor on a year's leave of absence for private research at the Center for Advanced Study in the Behavioral Sciences. The centre is an independent institution providing seclusion for internationally selected scholars in the social sciences. It is paid for by the Ford Foundation and located on a woody hilltop on Stanford University property. It has no other links with Stanford or any other university. The letter to President Nixon presented a rather exaggerated account of an arson attempt on the centre when it was deserted the previous weekend (Friday, April 24). It also described how as a result the fruit of a distinguished Indian anthropologist's 22 years research on a multi-caste village in southern India had gone up in smoke. The writer also speculated on the identity of the fire raiser or fire raisers, though the culprits were unknown and remain so.

Another American fellow at the centre, Professor J. Bunzel, who had been active against campus radicals at nearby San Francisco State University, denounced the incident on television as a personal attack by radical students against himself. He had not in fact suffered in the fire, which was rapidly controlled, causing damage to the studies of only three out of fifty fellows.

The principal victim, Professor M. N. Srinivas of the University of Delhi, has been most careful not to lay blame in any quarter, and certainly not on protesting students. The evidence is simply non-existent. There is evidence that the fires were caused deliberately (two took hold), but it seems unlikely that the arsonist will ever be traced.

In his letter of condolence to Professor Srinivas in the last week of April, President Nixon firmly pinned the blame on dissident student elements. "To say they are simply evil does not make them go away." As Professor Srinivas freely admits, this has made it difficult for him to reply to the President without discourtesy. He achieved it on April 29.

A week after the exchanges with the President, and after his remarks on May 1 about "bums burning up books", and when it emerged that the incident at Palo Alto centre had achieved a notoriety out of all proportion to its significance, a letter signed by 33 of this year's 45 resident fellows was sent to the local newspaper, the *Palo Alto Times*, attempting to put the record straight.

The damage to Professor Srinivas's original material has proved less catastrophic than first supposed. About two-thirds of the data burned can be reconstituted by emergency measures already in hand and the rest is being replaced from India, and a research assistant is being specially flown in to help the professor. "I feared he would have to spend a further decade reworking his material," the centre's acting director, Dr Preston Cutler, said last week. This can be avoided. Dr Cutler's ultimate concern has been damage to the centre's principles of operation. "Our self image has always been wide open, hospitable and safe." Now the centre has armed guards on duty 24 hours a day on its open gates.

POPULAR ENTOMOLOGY

Year of the Locusts

Washington is now facing with some surprise an infestation of the periodic cicadas—popularly known as the seventeen-year locusts. The last severe onslaught of these insects was in 1953, and this one is being borne with some fortitude because the next will not be until 1987. The locusts have an extended life cycle in which the females each lay some 400–600 eggs in the branches of trees during June, and shortly after the birth the worms burrow underground to remain there for the next sixteen years and ten months. The males emerge from the ground first, climb up into the trees, and begin to sing after some four to five days (a high pitched humming which now characterizes the countryside around Washington) to attract the later emerging females, which then lay their eggs to restart the cycle.

Unlike the more commonly known and feared virulent locust, the cicadas—about an inch and a half long with large translucent wings—are harmless to man and only indirectly harmful to vegetation. They do not eat the leaves of plants in their short lifetime above the Earth, and their only deleterious effect upon the local ecology is the damage caused to the branches of trees by the holes punched by the females during egg laying. Although this can be a serious commercial problem for orchards, it is not more widely damaging.

Although Washington can expect relief from locust infestation for more than sixteen years, some areas of the American continent will suffer each year, for there are seventeen "broods" of periodic locusts, one for each year. Each brood seems to be part of the same species. But because any particular brood appears above ground only in its characteristic seventeenth year, it is reproductively and ecologically isolated from the others. The cycle seems to be maintained with high regularity and the occasional stragglers which emerge a year too early or too late usually die. Also assisting their ecological isolation, the different broods have different preferred areas; this year's brood (number X) is the most numerous and the widest ranging, being found in an area bounded by the Great Lakes in the north, New York in the east, Virginia in the south and Mississippi in the west. Three other broods will also be seen in Washington (XIV, due in 1974; II, due in 1979; and VI, due in 1983), but none so fiercely.

All in all, locusts are probably a little more complicated than just a single species divided into seventeen yearly broods. More recently, it has been suggested that there may be some six distinct species of periodic cicadas, each existing as a pair-species. That is, each may have broods with both seventeen and also thirteen year cycles. In that case, there would be a chance for the different broods to mingle, presumably one year in 221, when the thirteen year and seventeen year cycles coincide—but this is not due again until the twenty-second century.

The seventeen year cycle of the locusts was known to the American Indians and was first studied before the beginning of this century—the first brood was numbered in 1893—but this system has been subjected to surprisingly little scientific study in recent years. It provides, after all, an unparalleled opportunity to study the differences which there may be between the gene pools of a single organism existing in different ecological niches, but with the unusual factor that these niches are not necessarily separated geographically, but may be only temporarily distinct. That is, apart from year to year differences in climate, some of the various broods have the same ecological environment.

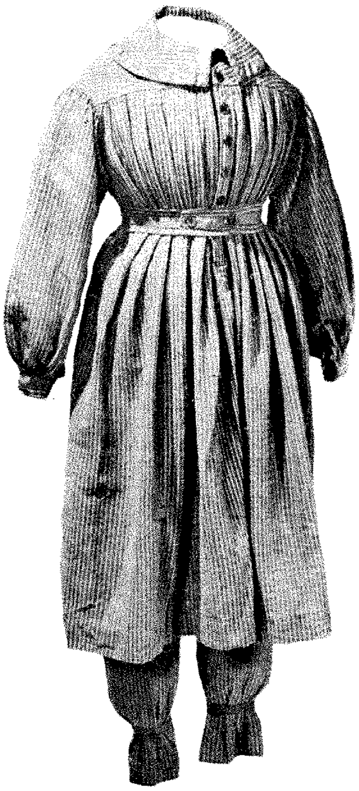
Another intriguing problem is that of how the insect maintains itself in what must be essentially a metabolically inert state for almost seventeen years. But perhaps it is not so surprising that little is known about how the locusts maintain their reproductive cycle with such unfailing regularity, for, after all, one research scientist can fit only two, at most three, locust cycles into his working lifetime.

FASHION

Swimming for Freedom

It is not just a matter of physical relief that the present 1920s look in women's clothing fashions has not been extended to swimming costumes. In any case, the evolution from the bathing costumes of those days to the contemporary garment demonstrates the metamorphosis of woman from the subordinate weaker sex to the "equal and active member of society" she is today. This at least is the thesis of Claudia B. Kidwell of the Smithsonian Institution, who has compiled a historical survey of the female bathing costume in the United States (*Contributions from the Museum of History and Technology*, p. 169. Smithsonian Institution Press, 1969).

It was the rise of the Christian Church that spelt the demise of the social aspects of bathing as practised by the Greeks and Romans, and it was not until the seventeenth century that women re-entered the water, and then only in small numbers. During the eighteenth century bathing became slowly more popular as the health-giving properties of water began to be emphasized, and by the 1800s women were being encouraged to swim. Bathing houses sprouted up wherever springs could be found, and bathing in the sea became an established pastime. However, throughout this period women paddled rather than swam and the bathing costume was designed more for adornment than use, besides which modesty demanded that it should cover every part of the female form.



Bathing dress of about 1855 (Philadelphia Museum of Art).

By slow degrees comfort as well as fashion crept into the styles until by the 1850s women were allowed to reveal themselves as bipedal, the original shapeless chemise-type gown giving way first to lace-edged pantaloons, and later to knee-length drawers worn beneath a long overdress. The skirtless bathing suit was next on the scene, but won greater popularity in Britain where bathing was segregated than in the United States where it was mixed. By the 1870s short-sleeved, low throated suits were being made and, as the century progressed and women grew more daring in the water, functional styles continued to advance. Costumes became more streamlined, trousers became kneelength, and by the beginning of this century freedom was on its way.

During the 1920s the swimming suit as opposed to the bathing suit gained ground, a trend accelerated by a growing advertising and ready-to-wear clothing industry. Women began to take the sport seriously, and though prejudice was rife a swimming costume almost recognizable as such began to take shape. When in the 1930s the desire to obtain a suntan forced costumes to become briefer and briefer, the marriage of style and utility was slowly but surely taking place. With the bikini the bathing costume has, of course, reached, if not its *ne plus ultra*, at least the end of an absorbing evolutionary track.

NEWS AND VIEWS

Swings and Ribosomes

ON the whole the followers of molecular biology have little difficulty in picking the winners. But occasionally they find themselves in the same invidious position as punters betting on a two-horse race contested by runners with comparable pedigrees and the same trainer. In the past two weeks just such a situation has developed over the question of which elements of the bacterial translation machinery recognize the different initiation signals in messenger RNAs that tell ribosomes where to start synthesizing proteins.

Undoubtedly in the first step of the translation of the information coded in a messenger RNA into the sequence of amino-acids in a protein the small 30S ribosomal subunit recognizes and binds to a specific initiator sequence in mRNA. Once this has happened formylmethionine, the initiating amino-acid, is carried by its specific transfer RNA to the complex, then the 50S ribosomal subunit is added and synthesis of the protein can begin. Clearly the specificity of this initiating process resides in the first step, the recognition of the initiator sequence by the 30S subunit. But only in the past six months has the degree of sophistication of this recognition process become apparent.

Last December (*Nature*, **224**, 957; 1969) Steitz reported that the three genetic messages which are contained in the RNA of the RNA coliphage R17, and specify the three phage proteins, begin with three different initiation sequences. The 30S ribosomes of *E. coli*, the host of these phages, obviously must be able to recognize these three start signals. Moreover, because the three phage proteins are not made in equimolar amounts in infected bacteria or cell free systems, the ribosomes must be able to discriminate between the three signals. Such discrimination, of course, provides cells with another point at which gene expression can be regulated, but how is this achieved?

One obvious possibility is that the ribosomes have permanently built into their structure the capacity to recognize specifically one particular initiation sequence. Alternatively a factor (or factors) might transiently associate with the 30S ribosome at initiation and temporarily confer on it the necessary specificity. This mechanism has the great advantage of flexibility. It does not force the cell to commit permanently its ribosomes to any particular start signal. Moreover, there is a precedent for mechanisms of this type. Sigma factors transiently associate with RNA polymerase and confer on it the ability to recognize particular sequences in DNA which signal where RNA synthesis should begin.

Knowing that *E. coli* ribosomes can discriminate between three initiation signals, and with the sigma factor precedent in mind, the stage seemed set for a series of elegant experiments proving that initiation factors regulate the selection of start signals in messengers. Of the three initiation factors in *E. coli* f3, the factor required for the translation of natural but not synthetic messengers in cell free systems, seemed the best candidate for the job. Some of the anticipated experiments have duly been done by Dube, Rudland and Steitz (see pages 820 and 824), but Lodish (*Nature*, **226**, 705; 1970) has well and truly upset the apple cart.

In brief, Dube and Rudland have shown that after infection with T4 phage the ribosomes of *E. coli* recognize the initiation signals of T4 mRNAs much more efficiently than they recognize start signals in *E. coli* or RNA phage messengers. Moreover, when initiation factors that can be washed off ribosomes from infected cells are added to washed ribosomes of uninfected cells, these ribosomes then behave as if they came from infected cells. In other words, after infection with T4 it seems that a new set of factors, specified by the phage, are made. These then displace the host cell factors and cause the ribosomes to recognize preferentially the start signals of T4 messengers, while restricting their capacity to recognize *E. coli* start signals. The new initiation factors subvert the host cell's synthetic machinery towards making phage protein. Rudland and Dube have gone some way towards isolating the new factors, and the next step is to characterize them.

With Steitz, Rudland and Dube have followed up their finding that T4 infection dramatically reduces but does not completely abolish the ability of *E. coli* ribosomes to bind to R17 phage RNA. Using factors and ribosomes prepared by Rudland and Dube in the system for isolating the R17 start signals, Steitz and her colleagues find that the factors from cells infected with T4 selectively change the capacity of *E. coli* ribosomes to bind to the three different R17 signals. Ribosomes with initiation factors from uninfected cells bind much more efficiently to the start sequence of the R17 coat protein than to the start signal of the maturation gene. By contrast, ribosomes with initiation factors from infected cells discriminate against the start signals of the coat gene; they bind with equal efficiency to both coat and maturation genes. The conclusion from these experiments must be that the initiation factors, not the ribosomes, determine the efficiency with which different gene initiation signals are recognized.

In *Nature* last week, however, Lodish reported equally clear cut experiments leading to precisely the opposite conclusion. He has measured the synthesis of coat, maturation and RNA replicase proteins of the RNA phage f2, a near relation of R17, in cell free systems primed with f2 RNA. In a cell free system from *E. coli*, the host of f2, all three proteins are made, but the amount of coat protein produced greatly exceeds the amount of maturation protein. A cell free system from *Bacillus stearothermophilus*, however, makes only maturation protein. Using mixed cell free systems with various components from *E. coli* and *B. stearothermophilus*, he shows convincingly that the source of the 30S ribosomal subunit uniquely determines whether three or one phage proteins are made. For example, a system containing *B. stearothermophilus* 30S subunits, *E. coli* initiation factors and 50S subunits makes only maturation protein. Conversely, if the 30S subunits come from *E. coli* all three proteins are made. Lodish also mentions that the ribosomes of *B. megatherium*, like those of *B. stearothermophilus*, will only translate the maturation protein gene.

Lodish's results point to the 30S ribosome as the determinant of the specificity of translation, while Dube and Rudland and Steitz's experiments indicate that the initiation factors play a part in messenger selection. But are *E. coli* and *B. stearothermophilus* infected with T4 phage really comparable? The messenger selection process involving initiation factors may be a specific feature of phage infection, a mechanism for sequestering the host's synthetic machinery for the production of phage protein just as sigma factors induced by T4 phage subvert *E. coli* RNA polymerase to making T4 phage RNA. On the other hand, it may be an example of a universal process for transiently programming ribosomes to recognize particular initiation signals.

In terms of cellular economy this is an attractive suggestion, but if it proves to be the case how can Lodish's experiments be explained? It would be particularly valuable to know how many R17 start signals can be recovered in Steitz's system using washed *E. coli* ribosomes and *B. stearothermophilus* factors. Lodish's experiments indicate that all three signals should be obtained, whereas if the initiation factors select start signals only that of the maturation protein should be found. Until further experiments have been done, you can pay your money and take your choice, or rather enjoy your prejudice.

PROTEINS

Through the Polarimeter

from our Molecular Biology Correspondent

IMPROVEMENTS in instrumentation during the past year or two have enormously increased the scope of optical rotatory dispersion (ORD) and circular dichroism (CD) measurements in protein chemistry. Aficionados of the technique will find several papers to engage their attention in the current literature.

In globular proteins, it is now a commonplace that Cotton effects, often of considerable magnitude, occur in the near-ultraviolet absorption bands of tyrosine and tryptophan. A systematic analysis of these effects has been attempted by Horwitz, Strickland and Billups (*J. Amer. Chem. Soc.*, **92**, 2119; 1970), who have followed up their previous papers on the optical activity of tryptophan and phenylalanine derivatives with a study of tyrosine, in relation particularly to ribonuclease, which shows a substantial Cotton effect associated with the absorption band of its six tyrosine residues. A series of simple derivatives studied in glassy solvents at liquid nitrogen temperature show fine-structure in both absorption and CD, which makes it possible to analyse the vibrational composition of the bands. The phenolic chromophore contains two transitions in the near ultraviolet, and Horwitz *et al.* have been able to make out the two corresponding vibrational progressions, one strong and one weak. Their positions are very solvent-sensitive, and it is therefore to be expected that in ribonuclease, which has three buried and three exposed tyrosine residues, there will be progressions arising from both classes (if both show enhanced optical activity). At low temperature, fine-structure appears, and with the aid of a curve-resolver, and only a modicum of imagination, bands representing the first members of three progressions can be discerned, two from internal, and one from the external, solvent-perturbed, residues. A large disulphide contribution extending to long wavelengths is also in evidence. Thus it is in principle possible to distinguish not only tyrosine from tryptophan components, but also internal from external ones, and the possibility arises of observing events around individual aromatic residues in globular proteins generally.

The remaining examples are concerned with some applications of ORD and CD in following changes in structure. Conjugated proteins, such as those containing haem, show complex systems of Cotton effects in the absorption bands of the prosthetic groups. Drucker, Campbell and Woody (*Biochemistry*, **9**, 1519; 1970) describe the Cotton effects of the cytochrome c_3 of three related sulphate-reducing bacteria. In spite of the low molecular weight of 14,000, these proteins are reported to carry three haem groups each. They have very large Cotton effects, particularly in the intense Soret absorption band. The results show that in spite of the considerable differences in amino-acid composition the three proteins are effectively identical in terms of the haem environment. On acid denaturation, there is a sharp diminution in the Cotton effects, and this can be used to follow the denaturation with great precision. Treating the process as a proton binding equilibrium, binding plots show that six protons are taken up on unfolding. Three of these are surmised to involve the haem-linked imidazole groups.

A number of workers have been much exercised to interpret the CD of intact membranes. This has, not surprisingly, proved difficult, because of the macroscopic nature of the material. Urry and Krivacic (*Proc. US Nat. Acad. Sci.*, **65**, 845; 1970) have analysed the effects of differential scattering of left and right-circularly polarized light—a phenomenon long known in optics—and have shown that it can in principle account for much of the distorted shape of the peptide Cotton effects observed in these systems. In spite of this kind of complication, however, it is occasionally possible to extract useful information from the CD of membranes. Glaser *et al.* (*ibid.*, 721) have, for example, found that the peptide Cotton effects, and therefore the protein conformation, do not change on treatment with phospholipase, which leads to the release of some 70 per cent of the total phospholipid. Moreover, the digestion is accompanied by the emergence of a series of resonances in the proton magnetic resonance

(PMR) spectrum, not evident in the intact membrane, and corresponding to the spectrum of extracted lipids in solution. By contrast, when membranes are heated, the breakdown of the protein structure can be followed by the change in CD, and this is associated with the appearance of the PMR spectrum of unfolded protein chains, but not of the methylene resonances of the fatty acid lipid chains, which evidently remain essentially immobile. It thus seems that the disordering of the proteins and of the lipids are independent and unrelated processes. Glaser *et al.* infer that the proteins are directly associated with only the minor fraction of phospholipids that are not destroyed by phospholipase, and are distributed in self-contained packets in a lipid bilayer matrix.

POLIO VIRUS

All or Nothing

from our Cell Biology Correspondent

At first sight the RNA coliphages have much in common with polio virus and its relatives. Both the RNA phages and the RNA viruses have a single stranded RNA molecule for a genome which also doubles as a messenger RNA. Moreover, the phage RNA certainly, and the viral RNA most probably, specifies a replicase enzyme for its own replication. But there the similarities end; the way in which the phage and viral proteins are made could hardly be more different.

The phages specify three proteins, their coat, their replicase and a maturation protein. Polio virus, on the other hand, has enough genetic information to specify about ten proteins, four of which are in the capsid and a fifth is the replicase. On the face of things polio virus is far more sophisticated than the coliphages, but compared with them it makes its proteins in a curiously unregulated fashion.

Both *in vivo* and *in vitro* the coat protein gene of the RNA phages is translated more efficiently than the other two genes. Apparently the coat protein molecules act as repressors for translation of the replicase gene while the maturation protein gene is initiated with a base sequence for which *E. coli* ribosomes have a comparatively low affinity. The net result is the preferential synthesis of coat, which one imagines confers a selective advantage on the phage, which needs 180 coat molecules but only one maturation protein to encapsidate one RNA molecule.

By contrast the translation of polio virus RNA, as the work of Baltimore and his associates has shown, is a profligate affair. The results of experiments carried out during the past several years suggest that the whole genome is translated into a single polypeptide chain which is then cut up to yield the specific viral proteins. By using three amino-acid analogues, Jacobson, Asso and Baltimore (*J. Mol. Biol.*, **49**, 657; 1970) now claim to have isolated for the first time this mammoth polypeptide. It has a molecular weight in excess of 200,000 daltons and, in the uniquely cumbersome terminology polio workers have saddled themselves with, is labelled non-capsid viral protein -00 (NCVP-00). Apparently the amino-acid analogues prevent NCVP-00 from being cleaved as it usually is before its synthesis is complete; the largest nascent polypeptides recoverable from cells infected with polio virus in the absence of analogues have molecular weights of less than 130,000.

Drawing on their own earlier work and that of others, and by isolating the various cleavage products of NCVP-00 and making tryptic peptide maps to sort out their relationships, Jacobson *et al.* have proposed the following scheme for the origin of polio virus proteins. NCVP-00 is envisaged as the common precursor which, when cleaved, yields NCVP-1, NCVP-2 and NCVP-X. Only NCVP-1 provides the proteins found in the virus. It is split to yield viral proteins VP1 and 3 of the procapsid as well as VP-0. VP-0 is envisaged as the precursor of the two other viral proteins VP2 and VP-4 which are generated as the procapsid matures. NCVP-2 and NCVP-X are presumably the precursors of the viral proteins involved in RNA replication and the switching off of host metabolism.

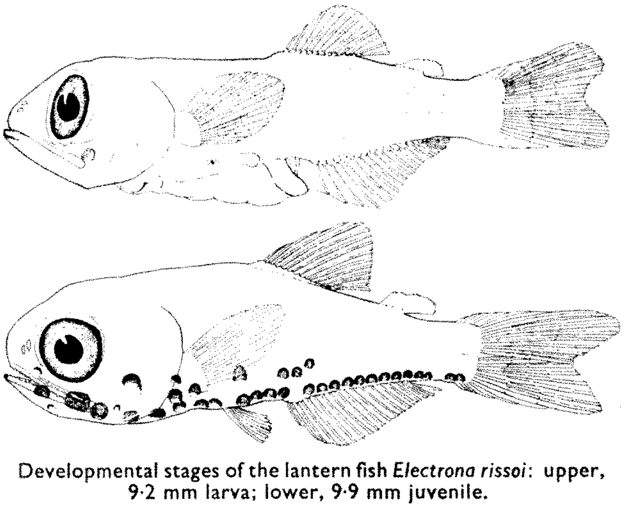
Apart from the problems of controlling the specificity of these cleavages the most surprising aspect of this process is that to make a coat protein polypeptide, polio virus apparently has to make all its other proteins. By comparison the RNA phages are models of cellular economy.

DEVELOPMENT

New Light on Lantern Fishes

from our Marine Vertebrates Correspondent

Lantern fishes, recently the subject of a valuable developmental study, are both abundant and widespread in the upper layers of the oceans, and therefore one of the primary forage foods for larger and commercially important oceanic fishes. In some cases densely shoaling species are fed on directly by tunas in the Pacific, and numerous lantern fish have been found in the food of the germon (*Thunnus alalunga*) in the Golfe du Gascogne.



Developmental stages of the lantern fish *Electrona rissoi*: upper, 9·2 mm larva; lower, 9·9 mm juvenile.

But the principal place of these myctophid fish in the feeding relationships of the ocean is at one remove, for they are preyed on by the smaller predators which are then eaten by the larger fish. They thus form a vital link in the food web of the seas. Lantern fishes are perhaps the most widely distributed family of fish in the world's oceans, represented by many species, which, in spite of some revisionary studies, are not fully understood. A recent important contribution to the better undertanding of the eastern Pacific species has been made by H. G. Moser and E. H. Ahlstrom (*Bulletin of the Los Angeles County Museum of Natural History*, Science No. 7, 1; 1970).

Moser and Ahlstrom have examined material collected during ten years of intensive and systematic surveys of the California Current Region off California and Baja California. They fully describe and illustrate the developmental stages of fourteen species of myctophid belonging to eleven genera, restricted to larvae in which the eyes are narrow (in the other major group the eyes are rounded). In spite of this restriction the work is of considerable value.

The first major contribution to the description of lantern fish larvae and young stages from an adequate series was that of Tåning, who in 1918 described several Mediterranean forms. This early lead was not followed until Pertseva-Ostroumova (*Akad. Nauk. SSSR Inst. Okeanologii Trudy*, **73**, 79; 1964) described the larvae of eighteen myctophid genera from the Pacific and Antarctic Oceans. Later she described the larvae of a further nine Antarctic lantern fishes. But Moser and Ahlstrom disagree with some of the identifications given by Pertseva-Ostroumova.

Moser and Ahlstrom's work can therefore be seen as a major advance in the identification of the larvae of this group of lantern fishes. It is now possible in this area to use these larvae as biological indicators of the water masses in which they were found, and Moser and Ahlstrom distinguish several major ecological assemblages off the Californian coast. This work will also improve knowledge of the ocean food web.

GENETICS

Chemicals and Mutations

from a Correspondent

THE Environmental Mutagen Society, founded last year as a result of the concern felt by many scientists about the potential mutagenicity of the ever increasing number of synthetic chemicals in the environment, has just held its first annual meeting. About 250 of the society's 450 members attended.

In his opening address, Dr H. Bentley Glass (State University of New York at Stony Brook) discussed many of the problems involved in the detection of the genetic effects of chemical mutagens in the human population and in the extrapolation to man of data obtained with experimental systems. He stressed the urgent need for more research to enable geneticists to evaluate the effects of such agents directly on man.

The problem of monitoring the human population for an increase in the frequency of genetic damage was also discussed by Dr J. Crow (University of Wisconsin, Madison), who pointed out that even if methods could be developed for the detection of an increase in the genetic load there might be great difficulty in determining the exact cause of the increase. The feeling that harmful genetic effects in man should be prevented by careful screening of man-made chemicals before they are released on to the market was reflected when various test systems were discussed. Several speakers considered the relationship between the mutagenicity and carcinogenicity of nitroso and other compounds.

An investigation of the cytogenetic effects of cyclohexylamine in *in vitro* studies on tissue culture of the rat-kangaroo and *in vivo* studies with rats was presented by Dr S. Green (FDA), and Dr J. Verrett (FDA) reviewed the striking teratogenic effects of cyclamates in the chick embryo.

Evidence was given for the mutagenicity of various man-made chemicals that pollute the environment, including ethylene dibromide (Dr F. J. de Serres and Dr H. V. Malling, Oak Ridge National Laboratory), methyl mercury (Dr C. Ramel, University of Stockholm), captan (Dr G. Fiscor, Western Michigan University, Kalamazoo, and Malling and de Serres), and sulphur dioxide (Drs R. Shapiro, R. E. Servis, B. I. Cohen and M. Welcher, New York University).

MINERALS

Mineral Wealth of Wales

from our Structural Geology Correspondent

THE exploitation of minerals and the economic geology of Wales were the theme of a University of Wales colloquium held on May 14 and 15 at Cardiff. It was appropriate that the meeting coincided with the announcement of new legislation affecting planning for mineral exploitation in Britain, and Mr Cyril Leigh (University College, Cardiff) pointed out that the current exploration boom in Southern Ireland could be directly related to analogous legislation in Eire.

In a stimulating contribution, Dr J. W. Baker (University College, Cardiff) defined the fundamental tectonic patterns of the Welsh Region, which he claimed had already been initiated by Late Pre-Cambrian times. He speculated on the relationship between these and deep-seated metallogenic provinces. In the context of the recent discoveries of workable mineral deposits in Eire, delegates discussed the possibility of a symmetrical tectonic pattern between Ireland and Wales, and consequently of mineral deposits yet to be discovered in the Welsh Region.

As an example of mineralization in the Caledonian Province, Dr W. J. Phillips (University College, Aberystwyth) discussed the lead and zinc mineralization in Central Wales. By the application of hydraulic fracturing in rock mechanics he showed that the mineralization process was instantaneous. The hydrothermal solution pressure built up until pre-existing tension fractures were suddenly dilated with the consequent upsurge of mineralizing solutions, producing breccias around which the lead and zinc sulphide phases were deposited.

The description of the various ore mineral associations in South Wales by representatives of the Wolfson Research Group (University College, Cardiff) ranged from the haematitic iron ores of Glamorgan (Dr R. A. Gayer), a branch of the mining industry suffering from severe competition from the massive importing of relatively cheap ore from the Commonwealth, through the lead, copper and zinc assemblages in the Carboniferous Limestones and Mesozoic strata of the Vale of Glamorgan (Dr C. M. L. Bowler and Mr G. A. Kingston) to the gold ores of Dolaucothi in Carmarthenshire. Work being carried out by Mr A. Pinches on microbiological and biochemical searching methods for the gold ores may well lead to an economic process of gold production from low grade ores and from gold which is difficult to extract by standard chemical processes.

Bulk mineral deposits also received their share of attention, and Professor J. G. C. Anderson (University College, Cardiff) showed that there are large deposits of sand and gravel beneath the coastal alluvial clays of the Severn Estuary. These largely occupy buried river channels overdeepened during the glacial period. In his contribution Dr R. G. Thurrell (Institute of Geological Sciences) explained the complex procedure required for the assessment of sand and gravel resources.

ELECTRONICS

Surface Wave Filters

A NOVEL development in integrated circuit technology is the use of acoustic surface waves to make cheap and effective frequency selective filters for television amplifiers. This latest application of the newly fashionable surface wave devices is a departure from their chief use as dispersive elements in radar systems. According to a report in the Mullard Research Laboratories (MRL) annual review, the new surface wave filter can be used instead of conventional resonant circuit filters in amplifiers operating at frequencies of a few tens of MHz. At such frequencies inductors become too big to be used as part of integrated circuits and an alternative way of providing frequency selective elements has to be found.

The new MRL filter relies on the interference between acoustic waves generated on the surface of a piezoelectric crystal to provide the necessary selectivity. It consists of a pair of transducers formed as geometrical arrays of thin gold electrodes on the surface of a piezoelectric chip; one to generate and the other to receive the waves. The geometric arrangement of the electrodes determines the way in which the waves interfere, so that by careful choice of the geometry the response of the filter can be tailor made to suit a particular amplifier circuit. Using computer optimization techniques the Mullard team has designed filters which have apparently worked well in a 37 MHz colour television receiver.

The filter is small enough to be used as part of an integrated circuit assembly, but there are still problems in the way of integrating it completely with a silicon chip. The other problem in making the new device commercially viable is to find a ceramic base which is more stable against changes in temperature.

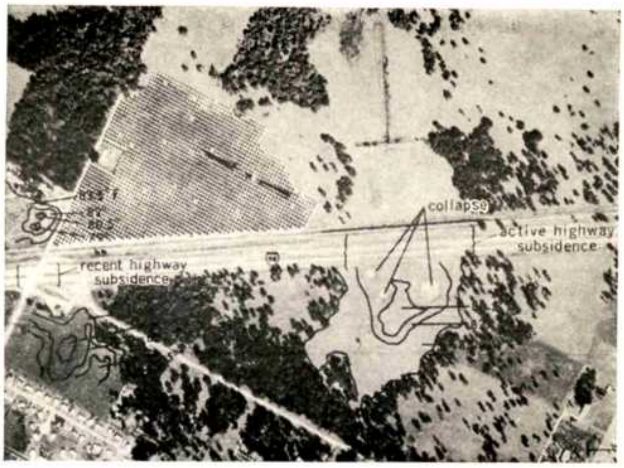
CATASTROPHES

Sources of Sinkholes

from our Geomagnetism Correspondent

NORTH AMERICA is subject to a remarkable variety of natural catastrophes—tornadoes, hurricanes, earthquakes and tsunamis, far from uncommon in one part of the country or another, are only the most devastating. Less spectacular, though equally frightening, expensive and, on occasion, fatal to those whom they afflict, are the minor geological perturbations which come under the general heading of land deformations.

"Sinkholes", or small localized regions of land collapse, are a case in point. The sudden formation of sinkholes is related to the existence of cavernous limestone, estimated to lie beneath about fifteen per cent of the forty-eight states which make up the continental United States. The most important of the areas where sinkholes are common is thus the "Great Valley" lying east of the Appalachian Mountains and extending from New York State to Alabama, Georgia and Florida, although land collapse of this type is not unknown in Kentucky, Tennessee and New Mexico.



A black-and-white print of a coloured infrared "thermal" map showing thermal contours superimposed on a photograph of sinkholes near Bartow, Florida. (Photo, US Geological Survey.)

From now on, however, sinkhole disasters should become less frequent because of progress made in the detection of potential sinkhole positions. The Bartow–Lakeside–Winter Haven region of Florida, an area particularly prone to sinkhole collapse, has been investigated by scientists from the US Geological Survey and the University of Michigan using airborne remote sensing devices. They found that collapsed regions and areas highly susceptible to collapse have anomalous thermal and moisture characteristics which are easily revealed by infrared detection devices. It is hoped that no further development will take place within clear disaster areas although, perhaps understandably, urban planners will have mixed feelings about the introduction of yet another restriction on their activities.

SIGNALS

Improving Radio Telephones

from a Correspondent

TRANSMISSION and reception of radio telephony at h.f. and v.h.f. provided the principal theme of the conference held by the Institution of Electrical Engineers in London from May 19 to 21. The topic for discussion was signal processing methods for radio telemetry, and there was no doubt that the most recent significant h.f. advance has been the Lincompex (linked compressor expander) system. This achieves a considerable saving in power in overcoming distortions due to multipath fading and noise. The principle of Lincompex is to bring all speech syllables to the same maximum amplitude (compression), so that all are transmitted at maximum power. A narrow band control signal is transmitted simultaneously, defining the degree of compression. At the receiver, the control signal is used to restore the received speech signal to its original form (expansion). Technical details and operational aspects of Lincompex were described in three contributions by a team from Marconi, GEC, STC, the Post

Office and Bell Telephone Laboratories. The first Lincompex system was introduced in 1967, and since then about 500 sets have been installed on point-to-point circuits.

Although radio telephony may suggest analogue techniques, about half the presentations dealt with digital signal transmission. Many aspects of signal processing are achieved more easily when the signal is digital, and analogue-to-digital conversion enables speech to be represented in this form. Digital transmission also brings the advantages of regeneration, tolerance to crosstalk and interference, and the opportunity for high security encoding.

Several contributors discussed the conversion of speech to digital form, including Mr R. M. Wilkinson (Signals Research and Development Establishment, Christchurch), who gave a subjective assessment of the various methods (all of which inherently give rise to distortion). He concluded, for example, that a six digit pulse code modulation system, compressed and then expanded, requiring a transmission rate of 40·6 kbits/s, gives a received speech quality which can be understood with almost complete relaxation in ideal transmission conditions. On the other hand, vocoder processing gives speech which is understood with slight effort, but the transmission rate is reduced to 2·4 kbits/s. Vocoder processing is important because 2·4 kbits/s can be transmitted in a single sideband channel normally used for conventional analogue speech transmission, although this is not achieved at h.f. without advanced signal processing. Four contributions dealt with adaptive methods of reception of such high rate data streams. This technique involves cascading the channel with a time varying network at the receiver, which serves to minimize dynamically the effects of channel distortion. But the usefulness of adaptive systems on h.f. channels was doubted by W. Struszynski (Marconi), who compared their performance with more simply realized systems.

Apart from the many problems of h.f. and v.h.f. radio telephony in analogue and digital form, the solutions of similar problems at higher frequencies were presented. G. L. Grisdale (Marconi) proposed a new application of swept frequency techniques for speech and data transmission from air to ground, via satellite, and R. C. Fitting (Motorola) presented design information and gave test results for a 2·8 Mbits/s tropospheric scatter data link.

ROCK MAGNETISM

Effects of Oxidation

from a Correspondent

THERE were some differences of opinion about marine magnetic anomalies and oxidized basalts at the annual meeting of the American Geophysical Union in Washington from April 20 to 24. The origin of the Mason–Raff magnetic lineations on the ocean floor has been explained by the Vine–Matthews hypothesis, which appeals to the normal and reversely polarized lava flows on the ocean floor. Direct confirmation of the hypothesis must wait until continuous coring can be extended beyond the sediments to the basalt flows. In anticipation of this, however, the basalts dredged from the mid-Atlantic ridge and away from it have been studied by Drs S. E. Haggerty (Carnegie Institution, Washington) and E. Irving (Observatories Branch, Ottawa) for an explanation of a certain peculiarity of the marine magnetic lineations.

The amplitude of the anomaly at the ridge is usually twice as large as the anomalies 5–10 km away (laterally). Farther from the ridge, the amplitude continues to decrease, but more slowly. Haggerty and Irving explained the initial decrease in amplitude on the basis of magnetic and petrological properties of basalts dredged from the Median Valley at the Mid-Atlantic Ridge (45° N) and those collected about 5 km away. They found that the opaque titanomagnetites in the Median Valley are unoxidized and carry an unusually large (10^{-1} gauss) natural remanent magnetization (NRM). Away from the ridge, however, titanomagnetites were oxidized ("maghaemitized") with a much lower (5×10^{-3} gauss) NRM. Haggerty explained the lack of oxidation in the Median Valley as the influence of the trapped sulphur volatiles: large concentrations of pyrite, pyrrhotite and chalcopyrite were found. Oxidation presumably starts as soon as the basalts are carried away from the ridge by sea-floor spreading. The NRM is partially destroyed and a chemical remanent magnetization (CRM) results which is much weaker than the initial NRM.

These results from dredged basalts were contradicted by laboratory oxidation studies performed on oceanic basalts by Drs M. Marshall and A. Cox (Stanford University). To be sure, they also found that the NRM can be destroyed by heating (to temperatures between 200° C and 300° C in air). But the new CRM that resulted from the laboratory oxidation was stable and strong and, for some specimens, several times larger than the original NRM.

There was doubt at the meeting as to whether the chemical composition and the crystallographic structure of the products of laboratory oxidation were the same as those relating to the maghaemites found by Haggerty in the naturally oxidized samples.

Professor K. Creer and his co-workers (University of Newcastle, England) presented magnetic data obtained from maghaemites or oxidized titanomagnetites, which were formed by heating to 200° C in air, after wet-grinding with water in a ball mill. Creer claimed that the preliminary wet-grinding process allows a more accurate simulation of the magnetic minerals found in natural basalts. But Creer was followed by Drs S. K. Banerjee and P. E. D. Morgan (Franklin Institute, Philadelphia), who reviewed all attempts at laboratory simulation of the magnetic minerals of natural basalts and pointed out their deficiencies. They pointed out that Mössbauer data obtained from Creer's wet-ground titanomagnetites show that even such materials undergo spurious changes in magnetic properties when oxidized, because the wet-grinding process produces ultrafine, superparamagnetic particles which later change their magnetic properties, not only because of oxidation, but also due to sintering and grain growth. It seemed to be crucial that in order to simulate natural oxidation, the starting laboratory samples must contain cation vacancies but no nuclei of rhombohedral haemo-ilmenite impurities. The presence of such impurities even in microscopic amounts (for example, stacking faults in the cubic titanomagnetites) leads to a kinetically preferred magnetite–ilmenite intergrowth, rather than the cation-deficient maghaemite found in nature.

Metabolic Adaptation in the Brain

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Enzyme adaptation in the brain involving substrates, hormones and neurotransmitters may participate in behavioural adaptation, consolidation in learning and central aspects of drug tolerance. This article is based on lectures given in October 1969 to the Oxford University Biochemical Society and to the Neurochemical Group of the Biochemical Society.

The brain has become the major means by which higher animals and especially man adjust to changes in their environment. Enzymatic and metabolic adaptation play large parts in the adjustment of most organs of the body to changed circumstances, and this article has been prompted by new data which demonstrate that metabolic adaptation occurs in the brain and is of much importance in cerebral functioning.

Cerebral Adaptation to Specified Substrates

The principal oxidizable substrate of the adult mammalian brain is subject to alteration by enzyme induction[1]. Limitation of glucose supply to the brain usually results in loss of consciousness; lowering the concentration of blood glucose by means of insulin causes coma, and other administered substrates do not then replace glucose in restoring full cerebral functioning[2,3]. But in starvation, when the carbohydrate reserves of the body are lost gradually and β-hydroxybutyrate becomes the principal oxidizable substrate in the bloodstream, the brain also gains the ability to utilize it[4]. This was found to occur after some weeks in man or after 2 days in the rat[1]. By this time the β-hydroxybutyrate dehydrogenase activity of the mitochondria in the rat brain had increased nine-fold. In man a cerebral respiratory quotient much below unity showed that compounds derived from fat were serving as major cerebral substrates.

Relatively normal consciousness was maintained during some weeks of starvation in man, although carbohydrate reserves would have been exhausted in 2 to 3 days even if the brain was the only organ which utilized carbohydrate[4]. Thus the brain not only oxidizes β-hydroxybutyrate but also derives essential energy requirements by doing so. The metabolic basis for this has been shown in the rat by demonstrating synthesis of adenosine triphosphate with β-hydroxybutyrate as the only oxidizable substrate in cerebral preparations from adapted animals[5].

This cerebral metabolic adjustment to changed bodily circumstances was a characteristic of β-hydroxybutyrate dehydrogenase rather than other enzymes[5]. The dehydrogenase was assayed when maximally activated, and so presumably this particular cerebral constituent had increased nine-fold while maintaining cerebral functioning during starvation. The mechanism is not at present specified, but there are resemblances to the way in which iron stimulates the synthesis of ferritin in the liver, and haematin stimulates the synthesis of the protein moiety of haemoglobin in reticulocytes or avian erythrocytes.

As for other enzymes of the brain, an increase in the activities of L-glutamate dehydrogenase[6] and tyrosine transaminase[7] was found when their respective substrates were added. Other examples of an increase in cerebral enzymes have depended on hormonal inducing agents—glycerol phosphate dehydrogenase[8] and Na, K-ATPase[9] stimulated by cortisol or cortisone, and lactate dehydrogenase[10] and adenyl cyclase[11] by adrenaline or noradrenaline, as discussed later. Cerebral examples thus seem to be available for two principal categories of enzyme induction[12]: one initiated by substrate or coenzyme and found in other tissues not to require synthesis of ribonucleic acid (RNA), and the other initiated by a hormone-like agent and typically needing additional RNA synthesis. The inducing agent, again, may affect either the synthesis or the breakdown of the component, RNA or protein, on which it acts; various principles may well be involved in the very different cerebral systems from which these examples are drawn (Fig. 1).

The ability to augment certain enzyme systems according to need has been regarded as an understandable part of the economy of simple organisms since the characterization of adaptive enzymes in bacteria[13]. The brain, also, can presumably be more compact and specific in function by not always possessing maximal enzyme activities for all the metabolic potentialities provided genetically. This implies regulatory processes conditioning genetic expression which in other organs can proceed by several mechanisms[12,14], some of which are considered later.

Adaptive Changes in Functioning

Augmentation of β-hydroxybutyrate dehydrogenase enables cerebral functioning to continue relatively unchanged when material supplies alter. Are comparable processes of metabolic induction involved when material supplies to the brain remain unchanged but cerebral responses alter? If so, evidence should be available for increased metabolic turnover in specified routes, or for increased synthesis of protein or of other necessary components. Table 1 summarizes four examples of such evidence.

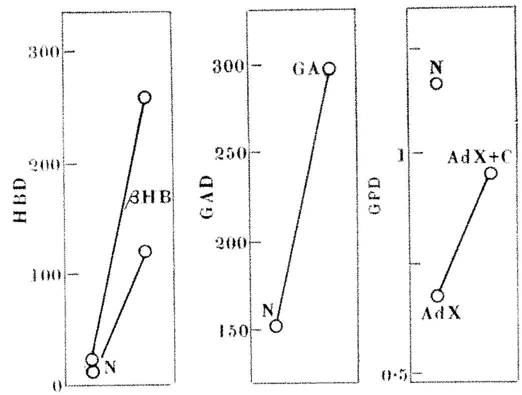

Fig. 1. Augmentation of enzymes in the brain of adult mammals by administration of a substrate or hormone. HBD, β-Hydroxybutyrate dehydrogenase (μmoles/g/h) on feeding rats diets which augmented circulating β-hydroxybutyric acid[1]; these animals (βHB) are compared with normal controls (N). The upper points give values for the enzyme activated with lecithin. GAD, Glutamic acid dehydrogenase (μmoles/g/h) of mice[6] with increased cerebral glutamic acid (GA) compared with normal controls (N). GPD, Glycerolphosphate dehydrogenase (μmoles/g/h) of rats[8]: animals were normal (N), adrenalectomized (AdX) and adrenalectomized and receiving cortisone (AdX + C).

Melatonin, an indole derivative which is formed exclusively in a particular part of the brain, the pineal, is involved in the first of these instances (second row of Table 1; Fig. 2). Melatonin controls diurnal and seasonal adjustments of activity in many species[15–17]. It has been found to modify the psychomotor activity (wheel-running)[18] of rats, and could cause an eight-fold change in the gonadal weight of hamsters[17]. The melatonin content of the pineals of rats varied seven-fold during the day according to the illumination incident on the animals[16]. The changes were found to be mediated by neural connexions which came from the eye and which terminated at the pineal and there liberated noradrenaline, which augmented hydroxyindole-O-methyltransferase, the final and rate-limiting enzyme synthesizing melatonin[19]. Two lines of evidence indicated this to be an enzyme induction. First, the enzyme was assayed in maximally activated conditions and had presumably itself increased several-fold in quantity and, second, the augmentation involved protein synthesis, being inhibited *in vivo* by puromycin[20] and in isolated pineal glands by cycloheximide[19].

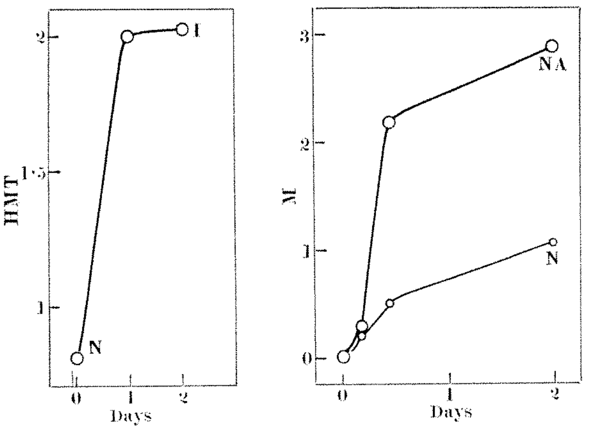

Fig. 2. Formation of melatonin (M; percentage yield from tryptophan) by hydroxyindole O-methyltransferase (HMT; pmoles/h) of the pineal gland of adult rats as a result of diminished illumination[20] (I), or of the addition of noradrenaline (NA) to cultured pineals[19]. N, Normal illumination or no addition.

Table 1. CHARACTERIZATION OF CEREBRAL ADAPTIVE PROCESSES

Process	Metabolic data
Maintenance of cerebral functioning in starvation and ketosis	Adaptive increase in mitochondrial β-hydroxybutyrate dehydrogenase
Adjustment of daily and seasonal rhythms by illumination: maintenance of certain gonadal functions	By pineal melatonin, the hydroxyindole-O-methyl synthetase for its formation being induced by noradrenaline
Development of tolerance to morphine on continued administration	Associated with increased incorporation of ^{32}P into phospholipids; inhibited by puromycin or cycloheximide
Sustained increase in cortical cell firing by surface-positive polarization	When established, persists after cooling the cortex to 20° C. Establishment blocked by cycloheximide or neomycin
Retention of certain induced behavioural changes:	
(*a*) Short-term memory	(*a*) Insensitive to inhibitors of RNA and protein synthesis
(*b*) Development of long term memory	(*b*) Blocked by acetoxycycloheximide and puromycin; actinomycin *D* depresses and could act by diminished protein synthesis

Metabolic data are chiefly from the rat; for references and further details, see text.

Phenomena of drug addiction and drug dependence have many characteristics which suggest that the changed behaviour of dependent animals is metabolically based. Development of tolerance to morphine in rats was found to be accompanied by changed oxidative and phospholipid metabolism in the brain itself (Table 1)[21,22]. When induced in acute experiments by intracarotid infusion of morphine, the development of tolerance was inhibited[23] by puromycin, cycloheximide or actinomycin *D*: enzyme induction thus seems to be involved in producing the state of tolerance. The cerebral metabolites most affected in the development of tolerance were phosphatidylserine and triphosphoinositide. The connexion between these and metabolic changes and the development of tolerance was demonstrated in the whole animal by several techniques. For example, there was slow reversion towards normal metabolism when addicting drugs were withdrawn[22], and some cross-tolerance was demonstrated at metabolic level between morphine, meperidine and methadone[21]. Mixtures of amphetamines and barbiturates, when causing long-term behavioural changes in experimental animals[24,25], were also concluded to act by blocking synthetic processes in the brain.

An imposed change in cerebral activity at electrophysiological level has shown properties which suggest it to be metabolically based (fourth row of Table 1)[27]. The firing of cortical cells in rats was accelerated four-fold by making the cortical surface electrically positive in relation to deeper structures[26]. After it had been imposed for about 10 min this change in rate of firing usually persisted for some hours after the positive polarization had been discontinued, and indeed it survived the cessation of electrical activity in the brain when this was brought about by lowering the animal's temperature. The immediate effect of the positive polarization in increasing cell-firing proved resistant to various treatments including the administration of inhibitors of protein synthesis, for example cycloheximide. Very significantly, however, these compounds prevented the continuance of the high rates of firing, which now reverted to their lower values when polarization was interrupted[27]. Persistence of the imposed change thus required new or augmented protein synthesis; the experiments did not indicate the nature of the protein so functioning, although an association with neurotransmitting agents would explain the observed action on cell-firing.

An interesting parallel is the state of knowledge about changes in cerebral activity induced by behavioural manipulations (fifth row of Table 1). In some of the best-devised and appraised cases[28–30] the immediate performances of animals in test conditions were unaffected by administrations of puromycin or of acetoxycycloheximide which inhibited protein synthesis in the brain by some 90 per cent. Performance on subsequent days was, however, below that of normal animals which had not received the compounds but had learned in the test conditions. The development of this difference was dependent on time; adopting the description of long term and short term memory to account for these performances, the administered compounds at first had no effect but blocked the process of consolidation which converted short to long term memory[30,31]. The incorporation of labelled precursors also suggested that cerebral protein and RNA fractions were altered when an animal's behaviour was caused to change in circumstances which as far as possible separated the behavioural performance from other bodily changes; in these conditions incorporation to cerebral DNA was not observed to alter[30].

Neurohumoral Agents in Metabolic Adaptation

When considering mechanisms, the first thing to note is that I have given six examples of alteration in the metabolic make-up of the brain when it is exposed to secreted humoral agents. Enzyme induction is indeed a major route of hormonal action generally. It can be involved when the brain is the target organ for compounds synthesized elsewhere: cortisol and triiodothyronine[32,33] have such actions; and it can be involved when the brain is the source of a hormone acting extracerebrally as when growth hormone from the pituitary acts on the liver[34], increasing there the incorporation of ^{14}C-leucine or ^{3}H-orotic acid. Induction of hydroxyindole-O-methyltransferase gave a purely intracerebral example of liberation of a neurohumoural agent and its induction of a critical enzyme. Do cognate phenomena occur more generally in the brain in relation to putative neurotransmitters? The investigations summarized in Table 2 suggest that this is often the case.

Table 2. RESPONSES OF ISOLATED PREPARATIONS FROM THE BRAIN

Agent applied	Cerebral system	Response
Electrical stimulation	Isolated tissues	Neural transmission[35]; cation movement[2,35] Release of acetylcholine[36], noradrenaline[37], serotonin[37], γ-aminobutyrate[38] Increased cyclic AMP[40]
Acetylcholine	Synaptosomes	Incorporation to RNA[57] and phospholipid[58] increased
Noradrenaline	Tissues, surviving or cultured	Adenyl cyclase increased[41]; lactate dehydrogenase induced[10]
Serotonin	Tissues, surviving	Cyclic AMP increased[40,59]
Histamine	Tissues, surviving	Cyclic AMP and adenyl cyclase increased[40,59]
Cyclic 3′5′-AMP	Cerebral dispersion or microsomal preparation	Phosphorylase kinase activated[41]; protein kinase increased[42,43]; incorporation to peptides increased[44]

When isolated cerebral tissues were used so that both the electrical stimuli and the humoral agents applied to them were controlled, excitation produced many responses in neural conduction, transmission, cation exchange and substrate utilization[2,35]. Further, the stimuli in various preparations released acetylcholine[36], noradrenaline, serotonin[37] and γ-aminobutyrate[38]. Often these compounds are regarded chiefly as agents for inhibiting or excitating postsynaptic cells, but Table 2 and the following data suggest that several if not all can be regarded also as agents of metabolic induction.

One route by which the induction may occur involves cyclic adenosine-3′5′-phosphate (cyclic AMP). Compared with other tissues, those from the brain have the greatest capacity for the formation[39] and breakdown[41] of cyclic AMP and their content of this compound can increase forty-fold when they are exposed to electrical stimulation and neurohumoral agents[40]. In many organs, cyclic AMP is intermediary in specified categories of hormonal action[39,41] and in cerebral tissues themselves, noradrenaline, serotonin and histamine each increase cyclic AMP[40,41]. Moreover, electrical stimulation alone also brings about this effect, concomitantly with the release of neurotransmitters[40]. Activation of phosphorylase kinase in the brain is brought about by cyclic AMP[41]; in this way catecholamines modify cerebral phosphorylase itself and so glycogen. Further examples of increase in cerebral protein kinases by cyclic AMP have been reported[42,43], and also a case of cyclic AMP increasing peptide formation in cerebral preparations[44].

Persistence and Sites of Metabolic Adaptation

The incorporation of labelled precursors into RNA and protein fractions from the brain shows them to have half-lives of a few hours or a few days[45,46]; this is comparable with the persistence of several of the adaptive changes of Table 1. There are clearly advantages in changes which persist for such periods: they allow concentrations of enzyme to follow a diurnal cycle, or to adjust to daily fluctuations in the sensory impressions or the foodstuffs which an animal encounters. Other alterations impressed on the brain last longer: some learned patterns of animal activity[24,28-31] and items remembered may persist for a lifetime. Commentators have invoked special reactions (for example, at the level of DNA[47]) to explain such phenomena; but several of the agents already involved in inducing metabolic changes which persist for a few days will, in other circumstances, produce much more enduring alterations in cerebral structure or enzyme concentration. The absence or presence of thyroxine between 10 and 15 days after birth caused differential and persistent changes in rat cerebral succinate dehydrogenase[48], which were not restored to normal by subsequent administration of thyroxine; the effect was selective, for four other enzymes did not undergo comparable changes. Neonatal deprivation of thyroxine also diminished glutamic acid decarboxylase and transaminase, and the sodium-potassium-activated adenosine triphosphatase of the rat cerebral cortex[49,50]. Again, the induced enzyme differences persisted even when thyroid hormones were given after 15 days. Cortisol accelerated myelination in rats[51] when given shortly after birth, and continued administration was not needed for maintenance of the induced myelin.

These examples are akin to morphogenetic induction, operating during relatively short periods of development, and three comments are relevant. First, as Wigglesworth has said, hormones merge imperceptibly into the chemical factors responsible for local differentiation in determination[52]. Second, much differentiation during development is irreversible; a hormone can at a suitable stage act as a genetic switch in deciding a route of development; even the availability of an amino-acid at a critical time can alter permanently the expression of genetic potential[52,53] in a given organism. Third, neural control over innervated organs can be complex: when amphibian limbs regenerate, outgrowth is first inhibited when neural connexions are made, and later selectively accelerated[54]. The corresponding situation in the brain would be for neural elements to mutually inhibit growth or differentiation in general, but to induce development selectively in relation to functioning. Thus the release of a neurohumoral agent may cause structural development of some permanence, as well as enzyme induction. Indeed, enzyme induction in such cases may form part of the resumption of a specifically delayed maturation.

It is important to specify the site at which a neurohumoral agent is pictured to cause enzyme induction or more permanent developmental change. The adaptation to utilize β-hydroxybutyrate is localized in mitochondria, but most of the mitochondria of the brain are likely to be equally exposed to β-hydroxybutyrate, and indeed a large proportion of cerebral mitochondria, independently of their cellular situation, must increase their β-hydroxybutyrate dehydrogenase activity to explain available data[1,4]. By contrast, as a neurotransmitting agent noradrenaline is active at highly localized sites, and perhaps the site of action of such agents in enzyme induction is similarly localized. The following cases of metabolic induction have indeed been found to be postsynaptic: hydroxyindole-O-methyltransferase in the pineal[16]; phospholipid metabolism on stimulating sympathetic ganglia[55] and protein and ribonucleic acid metabolism induced by neural excitation in the diaphragm[56]. It is further logical that the inducing function of a neurohumour should be exerted postsynaptically because in some cases the neurohumour is considered to be synthesized in the cytoplasm of the presynaptic terminals and subsequently taken up into vesicles; thus if induction occurred presynaptically it could have done so independently of excitation. That it does so in association with excitation is demonstrated by several examples in Tables 1 and 2. The persistence of many patterns of cell-firing suggests that the converse does not hold: not all excitation is associated with adaptive change. The responsiveness of postsynaptic cells in this respect changes during development and with hormonal conditions in the animal as a whole. Acetylcholine, which does not increase cyclic AMP as does noradrenaline[59], lacks this means of metabolic adaptation.

Further significance can now be seen in those structural and enzymatic arrangements at synaptic junctions which are known to localize the action of neurotransmitting agents. Presynaptic encapsulation of the agents in vesicles, oriented intercellular material at the synapse and enzymes frequently found extracellularly which inactivate the agent have been regarded as necessary to ensure localized changes in membrane potential or cell-firing when the agent is liberated. Equally, they can contribute to localizing an enzyme-inducing effect to a limited postsynaptic site. In the pineal, sensory input altered the production of melatonin by summation of qualitatively similar actions at numerous adrenergic terminals. In other parts of the brain, individual neural connexions are less uniform in site and function and the localization of the metabolic changes induced can be correspondingly specific. Indeed, enzyme induction so conditioned can be understood to play a part in the initial growth of the brain, by favouring particular synaptic connexions in a manner related to sensory input.

Adaptation and Metabolic Turnover

Knowledge of enzyme adaptation in the brain was sparse until quite recently. Two examples were quoted in 1966[2] but little could be said about mechanism. In 1968[28] Kandel and Spencer, describing phenomena of facilitation and plasticity in neural systems, commented that "nothing is known about enzyme induction in nerve cells". Adaptation of unspecified but possibly metabolic mechanisms is frequently encountered in studies of the brain. Concepts of adaptation and habituation feature in psychological interpretations of cerebral functioning, and an "adaptation-level theory" has been propounded[60] in systematizing experimental findings in behavioural studies. That the brain is capable of a multiplicity of enzymatically based adaptations is as significant as that it contains a multiplicity of cellular interconnexions. Indeed, the two properties are related, for metabolic adaptability makes for an economy of material and greater density of packing. It requires, however, a state of flux of the metabolic machinery itself. This is vividly seen in the high rates of protein turnover characteristically found in cerebral systems.

In summary, the enzyme make-up of the brain can change (i) in maintaining normal cerebral activities in varied conditions and (ii) when cerebral functioning alters in response to altered sensory input. These metabolic adaptations are necessarily persistent, the half lives of the categories of components involved being some hours or weeks, and in specific circumstances the induced changes can endure longer still. Some of the cerebral metabolic adaptations I have described are mediated by materials from the bloodstream and others by the neurohumoral agents which are produced intracerebrally and stored, liberated and act at synaptic structures. These latter agents can therefore cause metablic adaptation which is localized, in time and cellular site, in relation to cell-firing. They thus contribute to the persistence in the brain of a spatial pattern of metabolic change related to sensory input or to endogenous cerebral activity.

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Experimental Transmission of Feline Fibrosarcoma to Cats and Dogs

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An infectious C-type feline sarcoma virus (FSV), isolated from a naturally occurring fibrosarcoma of a domestic house cat, produces fibrosarcomas in kittens and puppies and induces cell transformation in feline embryo fibroblasts. Prenatal inoculation of cat and dog foetuses is particularly effective in tumour transmission. Postnatal dogs bearing FSV induced fibrosarcomas provide antisera suitable for detecting the feline C-type RNA tumour virus antigens in the complement fixation test.

MALIGNANT lymphoma in domestic cats, a relatively common feline disorder[1], is caused by a C-type RNA tumour virus[2–4]. It has been found that a feline fibrosarcoma, also caused by a C-type virus, is transmissible to kittens and puppies[5], and that the feline and murine C-type viruses share an internal soluble group specific antigen component[6]. Using rabbit antisera to ether treated feline leukaemia virus and immunogel diffusion, the presence of a group specific antigen in tissues from cats with spontaneous lymphoma was demonstrated[7]. We report here the transmission to cats and dogs of another naturally occurring feline fibrosarcoma, also associated with C-type particles, and the production in sarcoma bearing dogs of complement fixing antibodies suitable for detecting the feline C-type virus antigens.

Cat to Cat Tumour Transmission

The donor tumour was a recurrent subcutaneous fibrosarcoma obtained in March 1969 from the left flank of a five and a half year old male Siamese cat. The initial tumour was removed in November 1968, but it recurred and was irradiated with 1,000 rad in January 1969. The recurrent tumour, consisting of eight to ten firm 1·0 cm nodules located subcutaneously along the operative site, was used for electron microscopy, tissue culture and transmission studies.

The donor tumour was minced and diluted with tissue culture growth medium containing 10 per cent dimethyl sulphoxide and frozen at −70° C. After eight days storage, the tissue suspension was thawed rapidly in a 30° C water bath, centrifuged lightly (1,000 r.p.m. for 10 min) and resuspended in tissue culture growth medium to make a 10 per cent (w/v) tissue suspension. At laparotomy 0·5 ml. of this suspension was inoculated through the uterine wall into the shoulder region of six viable foetuses of a mixed breed cat at approximately 45–50 days gestation. Three apparently normal kittens were born 22 days after surgery; the other three foetuses were apparently resorbed *in utero*. Two of the three kittens developed large tumours in the shoulder region, first noticed when one was 45 and the other 71 days old (67 and 93 days following inoculation, respectively). These kitten tumours have been transmitted for three generations in 4–60 day old kittens using tumour homogenates similar to those described in the first passage (Table 1). Fibrosarcomas developed in fourteen of sixteen kittens after a latent period of 20–77 days. The longer latent periods usually occurred in the older kittens. The mother cat, from which foetuses had been inoculated with the donor tumour, was killed 100 days later; at necropsy several fibrosarcomatous nodules were found in the meso-ovarian ligament and in the uterine wall.

Cell-free virus concentrates were prepared from kitten tumours by a modification of the Moloney procedure[8,9]. (The final clarification step—low speed centrifugation—was omitted from the modified procedure.) Successful

Table 1. TRANSMISSION OF FELINE FIBROSARCOMA WITH TUMOUR HOMOGENATE

	Transplant generation								
	I	I	I	II	II	II	II	III	III
Cat to cat transmission									
Age at inoculation	*In utero*	Adult		2 weeks–2 months				4 days	
Number tumours/number inoculated	2/3	1/1		9/11				5/5	
Tumour latent period (days) measured from time of inoculation	67–93	100		34–77				20	
Cat to dog transmission*									
Age at inoculation	*In utero*								
Number tumours/number inoculated	6/6								
Tumour latent period (days)	18								
Dog to dog transmission†									
Age at inoculation	*In utero*	12–40 days	Adult	*In utero*	Newborn	3–7 weeks	Adult	3 days	6–8 weeks
Number tumours/number inoculated	28/28	0/8	1/2	6/6	8/12	8/21	0/4	2/3	0/4
Tumour latent period (days)	12–18	—	18	20	7–20	10–27	—	24	—

* The inoculum used was a homogenate from a second transplant generation kitten tumour.

† The inocula used for the first transplant generation were homogenates of puppy tumours that had been induced prenatally with kitten tumour homogenate; those for the second generation were from puppy tumours that had been induced prenatally in the first transplant generation; those for the third generation were from puppy tumours that were induced postnatally in newborn puppies in the second transplant generation.

sarcoma induction was obtained with Moloney concentrates in first passage in seven of eight, and in second passage in eight of sixteen, young kittens after latent periods ranging from 14–30 days (Table 2). In addition, one adult cat developed a fibrosarcoma at the site of intravenous injection of the virus concentrate after a latent period of 30 days.

The transmissibility to kittens of the original donor cat tumour was later confirmed by one of the authors (C.R.): all twenty-two newborn kittens in three serial passages developed fibrosarcomas following inoculation with homogenates and cell-free Moloney concentrates prepared from the original donor cat tumour or spleen.

Cat to Dog Tumour Transmission

Subsequent experiments revealed that puppies were also quite susceptible to sarcoma induction by transmission of cat tumour homogenates (Table 1) or cell-free concentrates (Table 2). Following the *in utero* inoculation of cat tumour homogenate into six dog foetuses at 45 days gestation, all six puppies developed large fibrosarcomas within two weeks after birth. Homogenates of these prenatally induced puppy sarcomas were similarly inoculated into twenty-eight foetuses of five pregnant bitches, at about 45 days gestation, and also into eight young puppies and two adult dogs. In addition, Moloney procedure cell-free concentrates of these same puppy sarcomas were inoculated into eight foetuses of two other pregnant bitches. All thirty-six foetuses had tumours at birth or within the first week of life. Only one of the postnatally inoculated dogs, an adult, developed a tumour. This was first noted after 18 days, and 5 weeks later it had completely regressed.

A second dog to dog transplant generation (Table 1), using inoculum from selected prenatally induced tumours, led to tumour "takes" after 7–27 days in six of six foetuses, eight of twelve newborn puppies, eight of twenty-one puppies age 3–6 weeks, and none of four adult dogs. A third transplant generation (Table 1), using inoculum from selected newborn induced tumours, produced "takes" after 24 days in two of three puppies, age 3 days, but no tumours resulted in the older puppies. In two adult dogs small tumours developed after 23 days which promptly regressed. Attempts to establish continuous dog to dog tumour transplants in weanling or older puppies are in progress. A cell-free Moloney concentrate prepared from a second transplant generation kitten fibrosarcoma induced fibrosarcomas at the site of intramuscular inoculation in four of five two-day-old puppies after an incubation period of 13 days (Table 2). Subsequent inoculations employing these tumours as the challenge material failed to induce tumours in four dog foetuses, fourteen puppies age 2 days to 2 weeks and one adult dog. The donor tumours failed to reveal C-type particles by the electron microscopy described here.

Puppy tumours induced by the cat tumour homogenates, as well as by the cell-free virus concentrates, have been subcultured and cells analysed for chromosome configuration; all proved to be dog karyotype.

The cat and dog tumours induced *in utero* or in newborns have shown progressive growth, often to large size (up to 250 cm^3). Because of the rapid tumour growth it has been possible to keep only a few of the kittens and puppies alive for more than several weeks after onset of tumour development. All the tumours in kittens and puppies were morphologically and microscopically similar to the original donor tumour. An extensive lymphocytic infiltrate was present in the uterine wall sarcoma from the initial mother cat and in the transplant tumours developing in puppies age 4–6 weeks, but not in those tumours developing in foetuses or newborn puppies. Multiple metastases have been noted in several of the kittens and puppies which were inoculated as foetuses or newborns but not in the older animals.

Occurrence of C-type Particles in Tumours

Numerous C-type particles were present in the fibrosarcoma from the original donor cat as well as in the spleen, bone marrow and mesenteric lymph node. Electron microscopic survey of tissues from fourteen kittens and two adult cats bearing either tumour homogenate or cell-free induced fibrosarcomas has revealed C-type particles in systemic distribution as follows: Tumour (16/16), bone marrow (12/12), mesenteric lymph node (11/11), thymus (7/7), spleen (6/8) and submaxillary gland (3/3). Equally numerous C-type particles were present in the same tissues from two puppies inoculated prenatally with kitten tumour homogenate. Fewer C-type particles were found in four dog tumours induced *in utero* and in newborn puppies by homogenates of prior prenatally induced dog tumours, and also in two dog tumours induced *in utero* by cell-free concentrates of prenatally induced dog tumours. C-type particles were not seen, however, in the tumours of two puppies that were inoculated when two days old with kitten tumour cell-free concentrate, nor in one of the six week old puppies bearing a transplant of a prenatally induced dog tumour.

A fibroblastic outgrowth from one second transplant generation kitten tumour has been maintained in tissue culture through four passages. C-type particles were observed at the fourth passage and a cell-free Moloney concentrate of the third passage cell pack induced a sarcoma two months after inoculation into a 15-day-old kitten. This tumour also contained C-type particles.

Partially purified Moloney concentrates of the experimentally induced feline and canine tumours induced cell transformation within 3–5 days in secondary monolayer cultures of feline embryo fibroblasts (P. S. S., M. B. G., and R. J. H., manuscript in preparation). The transformed cells were fibroblastic and refractile with parallel and criss-cross orientation; the latter was generally not readily obvious. Refractile round cells were also found in these cultures. Serially transmissible focus forming virus was also readily recovered from monolayer cultures of transplanted feline sarcomas.

Table 2. TRANSMISSION OF FELINE FIBROSARCOMA WITH CELL-FREE MOLONEY CONCENTRATES

	Passage I				Passage II	
	Cat to cat transmission*					
Age at inoculation	1–23 days	Adult			7 days	1–2 months
Number tumours/number inoculated	7/8	1/1			5/8	3/8
Tumour latent period (days) measured from time of inoculation	15–30	30			22–27	14–24
	Cat to dog transmission†					
Age at inoculation	2 days					
Number tumours/number inoculated	4/5					
Tumour latent period	13					
	Dog to dog transmission ‡					
Age at inoculation	*In utero*	2–12 days	1–2 months	Adult		
Number tumours/number inoculated	8/8	0/12	0/5	0/3		
Tumour latent period	9–12	—	—	—		

* The inocula used for the first and second cell-free tumour passages were prepared from the first and second transplant generation kitten tumours.

† The cell-free inocula used were prepared from a pool of two second generation kitten tumours (litter mates).

‡ The eight puppy tumours induced *in utero* developed after the inoculation of a cell-free concentrate prepared from prenatally induced puppy tumours of Table 1 (containing C-type particles). The same inocula failed to induce tumours in three young puppies and one adult dog. Cell-free inocula prepared from puppy tumours (containing no detectable C-type particles) that had been induced at two days of age with the second generation kitten tumour cell-free concentrate failed to induce tumours in the other postnatal dogs. Homogenates prepared from these identical puppy tumours (induced by the same kitten tumour concentrate) also failed to induce tumours in four other prenatally inoculated puppies (not shown).

Table 3. DETECTION OF FELINE RNA TUMOUR VIRUS COMPLEMENT FIXING ANTIGENS, ANTIBODIES AND C-TYPE PARTICLES IN DOGS AND CATS

A. Dogs

	Complement fixing antibodies against feline RNA tumour virus antigens*	Antibody titre range	Complement fixing of the feline RNA tumour virus group in tumour and/or spleen	Antigen titre range	C-type particles in tumour
Cat to dog sarcoma transplants *in utero*	0/3		2/3†	1 : 8 – 1 : 16	2/2
Cat to dog sarcoma cell-free passage age 2 days	1/4‡	1 : 40	3/3	1 : 2 – 1 : 16	0/2
Dog to dog sarcoma transplant *in utero* of prenatal induced sarcoma	1/6	1 : 40	11/12§	1 : 16 – 1 : 32	2/2
Dog to dog sarcoma cell-free passage *in utero* of prenatal induced tumour	Not tested		5/6	1 : 8	2/3
Dog to dog sarcoma transplant postnatal of prenatal induced tumour	12/20‖	1 : 40 – 1 : 80	1/3	1 : 16	3/4¶
Normal dog	1/4	1 : 40	0/1		Not tested

B. Cats

	Complement fixing antibodies against feline RNA tumour virus antigens*	Complement fixing antigens of the feline RNA tumour virus group in tumour and/or spleen	Antigen titre range	C-type particles in tumour
Feline lymphoma, naturally occurring	0/8	5/5§	1 : 4 – 1 : 16	4/8
Feline sarcoma, naturally occurring	0/5	2/5	1 : 2 – 1 : 4	1/5
Cats with FSV sarcoma transplants	0/6	6/6§**	1 : 2 – 1 : 8	10/10
Cats with FSV sarcomas, cell-free passage	0/5	3/5	1 : 2 – 1 : 8	6/6
Normal cats	0/10	1/10§	1 : 2	0/7

* Antigens tested against included extracts and/or cell-free Moloney concentrates from four prenatally induced feline sarcoma virus puppy sarcomas, from feline embryo fibroblasts cell packs infected with feline leukaemia virus, and from the spleen of a cat with spontaneous lymphoma. C-type particles were present in each of these tissues used for antigen preparation.

† CF reaction positive after ether treatment of antigens.

‡ This positive dog serum was used at a 1 : 10 dilution as the standard antisera for detection of the RNA tumour virus antigens in the tumour and/or spleen extracts shown.

§ The standard dog antiserum generally reacted in higher titre with antigens prepared by the Moloney procedure than with aqueous extracts.

‖ Most of these dog sera reacted in equal titre to either aqueous extracts or Moloney concentrates prepared from a prenatally induced dog tumour.

¶ The three positive tumours are from newborn inoculated puppies; the negative tumour is from a puppy inoculated at 6 weeks of age.

** Complement fixation reaction negative after ether treatment of antigen.

Serological Detection of Tumour Virus Antigens

Preliminary serologic studies indicate that dogs bearing feline sarcoma virus induced fibrosarcomas may provide a heterologous host system for production of antibody suitable for detecting the feline RNA tumour virus antigens in the complement fixation test (Table 3*A* and *B*). Ten per cent aqueous extracts, sonicated and clarified, were prepared from tumour and spleen of kittens and puppies with transmitted sarcomas, from cats with naturally occurring lymphoma and sarcoma, and from feline embryo fibroblast cell packs infected with feline leukaemia virus. Individual dog sera at 1 : 10 dilution were tested for complement fixation antibody versus a 1 : 4 dilution of these tissue extracts (Table 3*A*). Sera from one tumour bearing puppy inoculated at two days of age showed good complement fixation activity and did not show significant reaction with normal cat or dog tissue. This dog serum has been used for detecting feline RNA tumour virus complement fixation antigens directly in spleen and tumour extracts from cats with naturally occurring lymphoma or sarcoma (Table 3*A*) and in feline embryo fibroblast cultures following *in vitro* propagation of feline leukaemia virus. (P. S. S., R. J. H., and J. Baskar, manuscript in preparation). Sera from eighteen other puppies bearing tumour transplants for 4–6 weeks are being tested at weekly intervals and several have developed sufficiently high titred antibody responses to be useful for detection of antigen in feline tumour tissues and in infected tissue cultures. The specificity of the complement fixation reactions for either virus envelope antigens and/or internal group specific antigens is still undetermined, but some of the sera react with soluble ether treated antigens and are therefore presumably detecting group specific antigens. Using the canine serum we have detected the feline RNA tumour virus antigens in a total of sixteen cats bearing tumours (C-type particles were in fifteen) having either experimentally induced sarcoma or naturally occurring lymphoma or sarcoma (Table 3*B*). None of the sera from these cats has revealed complement fixation antibodies to these antigens. This apparent immunologic tolerance for homotypic antibody production may reflect vertical transmission and embryonic antigenic expression of the feline RNA tumour virus genome in the cat, an eventuality that might be expected from recent observations of natural tolerance in mice and chickens to the group specific antigen of their respective homotypic C-type genomes[10,11].

It is clear from the transmission experiments with cell-free concentrates in kittens and puppies and the *in vitro* transformation of feline embryo fibroblasts that certain naturally occurring sarcomas of domestic cats contain competent, fully infectious feline sarcoma viruses. The *in utero* passage of virus containing tumour cells and/or cell-free concentrates into kittens and puppies may offer a particularly useful method for *in vivo* propagation of highly tumorigenic sarcoma virus. The newborn host may also be tolerant to the infectious virus, thus accounting for the high potency and remarkably short latent period of the virus when transmitted to newborn kittens and puppies. Cell lines derived from tumours in these animals also provide potent sarcoma inducing preparations. By contrast, older cats and dogs have proved to be much less likely to develop tumours and the few tumours that did occur were of smaller dimensions and underwent regression in several adult dogs. In spite of the absence of detectable C-type particles the detection of antigen in several tumours that had been induced in two day-old puppies indicated that these tumours may contain the internal nucleocapsid group specific antigen (refs. 7, 12 and unpublished data of R. J. H., M. B. G., P. A. and J. D. E.) of the feline virus. The recent development of complement fixation tests for the antigens of the feline C-type RNA virus, and of sensitive *in vitro* assay systems for infectious virus[11], should make possible sero-epidemiologic studies to determine the natural history of the feline RNA tumour virus.

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Sexual Differentiation of the Brain: Effects of Testosterone on Brain RNA Metabolism in Newborn Female Rats

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RNA metabolism in two regions of the amygdala and anterior hypothalamus of the brain of the newborn female rat responds to testosterone administration quite differently from that in the brain as a whole. These two regions may represent primary sites for the action of this hormone on the brain.

IN adult male rodents, manifestations of sexual differentiation under the influence of the testis, for example, the acyclic pattern of gonadotropin release, appear early in development[1-3]—for the rat, in the first week after birth. The normal course of male behavioural and neuroendocrine development can be simulated in the female by the administration of testosterone propionate during this sensitive developmental period. Several lines of evidence[1-3] support the fact that the major sites of these differentiative processes lie within the central nervous system rather than in peripheral structures. In the brains of male and female animals many experiments have demonstrated the presence of neural receptors for the sex hormones which are involved in the activation of behavioural[4] and neuroendocrine[5,6] control mechanisms. Despite the large amount of experimental evidence that these and other studies have provided, however, there is limited information on the anatomical localization of sites at which the sexual differentiation of the brain occurs, and there are no observations that might relate to the biochemical basis.

We began with the assumption that the early effects of testosterone in the brain would involve enhanced RNA synthesis in differentiating areas and we used autoradiographic methods to examine the acute effect of testosterone on the incorporation of ^{3}H-uridine into the brain RNA of the newborn female rat. A similar approach to cerebral differentiation has recently been outlined by MacKinnon *et al.*[7] although the results did not concern hormonal effects.

We now demonstrate that testosterone reduces significantly the incorporation of uridine into brain tissue in general, but in two localized regions—the anterior hypothalamus and the amygdala—this reduction is not evident. These two regions of the brain have been implicated by others in functions of the central nervous system related to sexual activity and gonadatropin release. Our experiments suggest that they are directly involved in sexual differentiation.

Eight female Sprague-Dawley rats, 2 days old, were obtained from two litters and were divided into two groups of four. To minimize the effects of stress and handling, the cages containing the two litters with their mothers were brought into the laboratory at the end of the day preceding the experiment and the young were removed from the cages and placed in a container at 37° C early on the day of the experiment. Experimental animals were injected subcutaneously with 50 µg testosterone dissolved in 20 µl. propylene glycol. Controls received 20 µl. propylene glycol. After 3 h (a period chosen by analogy with results[8] for the time-course of action of testosterone on RNA synthesis in the seminal vesicles of the rat) each animal was injected with 250 µCi ^{3}H-uridine (specific activity about 2 Ci/mmole, in 10 µl. physiological saline). Exactly 1 h later the animals were cooled in ice for 5 min and then decapitated. The roof of the cranium was quickly cut away and each head was then placed in ice-cold Carnoy's fixative. These operations were carried out alternately on animals from the two groups. The heads were kept in the ice-cold fixative for 6 h. Brains were then dissected out and placed in absolute ethanol at room temperature for 12 h. The ethanol was changed every 4 h. The tissues were cleared in toluene for 3 h with changes of the solvent every hour. Then they were placed in paraffin (m.p. 45°–55°) at 59° with three changes at 1 h intervals, and embedded in the same medium. Each complete brain was serially sectioned transversely at 7 µm intervals.

The sections were mounted in sets of twelve on slides that had been treated with albumin; paraffin was removed in xylene and washing with a succession of alcohols followed finally by running deionized water ensured removal of all soluble radioactivity. The slides were dipped into Kodak 'NTB-3' emulsion kept at 43° C in complete darkness. They were drained, dried at room temperature for 3 h, then stored at 2° C in air-tight, light-proof boxes containing small amounts of 'Drierite' enclosed in paper packages. After 1 week, the slides were developed in 'Dektol' at 18°–20° C for 2 min, rinsed in deionized water, then fixed in Kodak rapid fixative for 5 min followed by a further rinse in deionized water for 15 min. The sections were stained by standard procedures with chrome–alum haematoxylin, counterstained with metanil yellow and, after being taken through a series of alcohols to xylene, were mounted in 'Permount'.

Specific regions of each brain were selected for counting silver grains (Figs. 1*a*–*d*). Grain counts were made at × 1,200 magnification under oil immersion with the aid of a net reticule placed in one eyepiece of the binocular. Most of the label in our experimental conditions was associated with the cell nuclei. For each given area, ten to eighteen sections were counted at intervals of two or three sections. The grains per nucleus were counted for fifty cells per section, as dictated by the alignment of the reticule. The results obtained by one observer were confirmed by a second working without knowledge of the experimental group from which the slides were derived.

For the purpose of direct radioassay of incorporated ^{3}H-uridine into brain tissue, the procedure was identical up to the point of transfer of the brain from the fixative into absolute alcohol. The brain was then homogenized in this solvent and insoluble material sedimented by centrifugation, then re-extracted five times with 70 per cent ethanol. The solid residue recovered from the last extraction was then dried for one week over phosphorus pentoxide *in vacuo*, and a weighed portion dissolved in hyamine. The hyamine solution was mixed with 15 ml. scintillation fluid containing 2,5-diphenyloxazole (5 g) and 1,4-*bis*-2-(5-phenyloxazolyl)benzene (0·3 g) per 1. of toluene, and its ^{3}H content assayed in a Packard liquid scintillation spectrometer. Heavy quenching by hyamine and yellowing of the samples made it necessary to ascertain the efficiency of counting of each sample by subsequent addition of $0{\cdot}89 \times 10^5$ d.p.m. ^{3}H-toluene (50 µl.) to

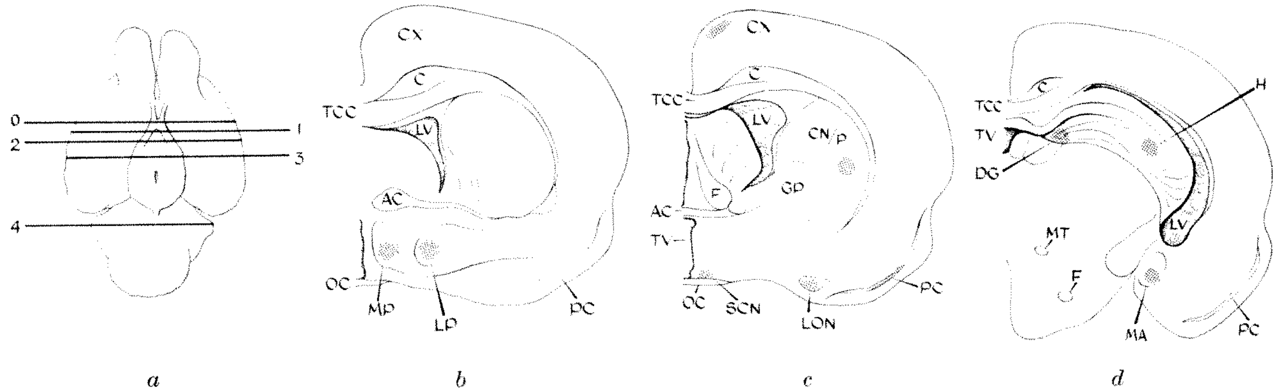

Fig. 1. Regions of the brain of the newborn female rat selected for autoradiographic measurements of incorporation of ^{3}H-uridine. *a*, General ventral aspect of the brain indicating levels of sectioning (1–3) represented in *b*, *c* and *d* respectively, and a cerebellar section (4), not shown. The line "O" represents a point of reference, the anterior limit of the optic chiasma. The stippled areas in *b*, *c* and *d* indicate areas selected for counting silver grains. AC, Anterior commissure; C, corpus callosum; CX, cortex; CN/P, caudate nucleus-putamen; DG, dentate gyrus; F, fornix; GP, globus pallidus; H, habenula; LV, lateral ventricle; LON, lateral olfactory nucleus; LP, lateral preoptic area; MA, medial amygdala; MP, medial preoptic area; MT, mamillothalamic tract; OC, optic chiasma; SCN, suprachiasmatic nucleus; TCC, truncus corporis callosi; TV, third ventricle.

each sample followed by re-assay. All assays were conducted in conditions which gave results well within 99 per cent confidence limits.

To demonstrate that the radioactivity incorporated into the tissue was in fact present in RNA, the histological sections and the extracted tissue residues were treated as follows. Before being dipped in the photographic emulsion they were exposed for three periods, each of 1 h, to a solution of bovine pancreatic ribonuclease (Mann, 5× crystallized) in water (0·3 mg per ml.) alternating with 1 h periods of rinsing in running deionized water. The sections were finally rinsed overnight in running deionized water, then dipped into the photographic emulsion. Subsequent handling was identical with that for other sections. Residues of brain tissue after homogenization and extraction with alcohols were treated with ribonuclease analogously. Weighed samples were triturated with the enzyme solution (1 mg per ml. water: about 0·5 ml. solution per mg tissue residue) and allowed to stand at room temperature for 1 h. The residue was then precipitated by centrifugation, the supernatant enzyme solution removed, and the sediment washed by resuspension in distilled water followed by recentrifugation. This was repeated twice and the final washed residue was again dehydrated with alcohol before radioassay of weighed, hyamine-solubilized portions. The enzyme treatment removed 80–90 per cent of the label and no significant differences in the residual label could be detected between experimental groups.

It was essential that the brains were kept as close as possible to 0° C while being exposed within the cranium and during fixation. Otherwise, great variations in grain counts were observed between animals of the same experimental group, and different areas of the brain showed wide and irreproducible fluctuations of incorporated radioactivity, probably because of enzymic breakdown of newly synthesized RNA which may occur to varying degrees in non-cooled tissue.

The cortical neurones, and in particular those of the lateral olfactory nucleus, were the most heavily labelled (Fig. 2). All other areas, including the medial preoptic area and medial amygdala, were more uniformly labelled, the grain intensity being different from that in the cortex and lateral olfactory nucleus. (Note that the cell nuclei of the lateral olfactory nucleus are probably the largest of all neuronal nuclei in animals of this age.) The incorporation of label (Fig. 2) is significantly ($P = 0{\cdot}05$–$0{\cdot}01$) lower in nine of the eleven areas studied in the testosterone-treated animals than in the controls. For the two exceptional areas, the medial preoptic area and the medial amygdala, the mean values in the testosterone-treated group were slightly higher than in the propylene glycol treated controls, but the difference between the two groups in these two areas was not significant. An analysis of variance of all results showed that the differential response (testosterone-treated compared with control) in the medial preoptic area and the amygdala differed significantly ($P < 0{\cdot}01$) from the response of other areas of the brain.

These results suggest that testosterone generally diminished uridine incorporation throughout the brain, the medial preoptic and amygdaloid areas behaving exceptionally. Possibly, however, the nine limited areas in which less incorporation was observed were not representative of the behaviour of the brain in general. To rule out such a possibility, the incorporation of labelled uridine was studied by direct tritium assay of the insoluble residue of the individual whole brains of groups of animals treated exactly as in the histological experiments. Each experimental group comprised eight newborn females. The mean

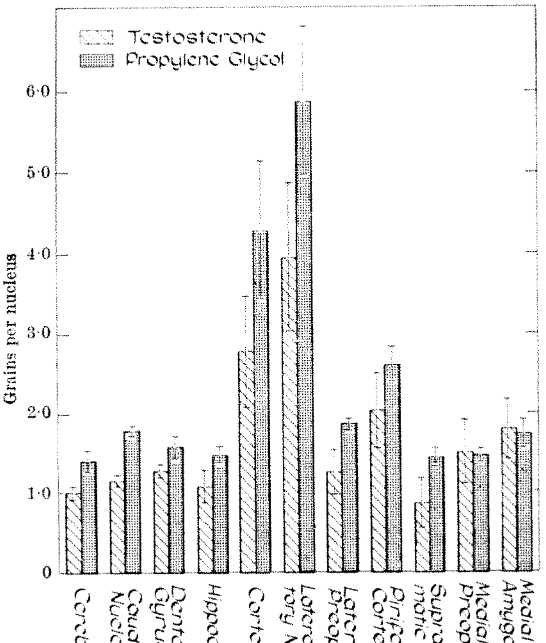

Fig. 2. Regional distribution of labelling of the brains of neonatal female rats by incorporation of ^{3}H-uridine, with and without previous treatment with testosterone. Each bar represents the mean grain count per cell ($\pm$ *S.D.*) in a given area for four animals. The value for each animal was the mean of the grain counts per cell over a number of histological sections (see text).

specific activity (±*S.D.*) of the RNA-containing residue of the brain tissue in the testosterone-treated group was 8,444±1,698 while in the control group it was 10,511±1,543. These values are different with a level of significance ($P=0·05–0·01$) of the same order as that established by the autoradiographic measurements, with the exception of those for the medial preoptic and medial amygdala, which must therefore differ from the rest of the brain in their response to testosterone.

The basis of the action of testosterone on nucleic acid metabolism in the neonatal brain is obscure. It may involve the activation by testosterone of a number of peripheral metabolic systems that take up uridine so that less of the labelled material is available to the brain. Fujii and Villee[9] found enhancement of RNA synthesis in the liver of immature rats following testosterone administration, but preliminary attempts on our part to account for the results found in the brain in terms of enhanced hepatic RNA synthesis have been inconclusive. A direct general effect of the hormone on some pathways of uridine metabolism in the brain, the result of which is overridden by a more specific type of response in the medial preoptic and amygdala, is also a possibility.

The divergence of the results obtained in the medial preoptic and amygdaloid areas from those found in the brain as a whole is of special interest. If, for whatever reason, less ^{3}H-uridine is available for the brain of the testosterone-treated animal than for the control, the failure of this effect to be registered in these two areas can best be explained as a specific local enhancement of incorporation which would be expected in neurones undergoing sexual differentiation. But even this interpretation is necessarily tentative. For example, we do not overlook the fact that measurements made by autoradiography cannot reflect dynamic aspects of nucleic acid metabolism and both synthesis and degradation of RNA may be affected. Numerous other observations make it reasonable to suggest, however, that either or both the preoptic area and the amygdala is intimately involved in the process of sexual differentiation of the brain. Implantation of pellets of oestradiol into the medial preoptic area of the oophorectomized rat reinstates sexual behaviour[10]. Lesions in the preoptic area cause disruption of cyclic control of gonadotropin release[11–14] and of normal female sexual behaviour[15,16]; electrical stimulation of this area can induce ovulation in the normal female[17,18] but not in the female treated neonatally with a single injection of testosterone propionate adequate to abolish both cyclic behaviour and female receptivity[19]. Cyclicity is also abolished by the severance of neural connexions between the medial preoptic area and the median eminence region[20].

Lesions in the amygdala were reported many years ago to result in changes in aggressive behaviour and a greatly increased interest in sexual activity[21,22]. In the infant rat, bilateral lesions in the amygdala[23] or transection of the stria terminalis[24], a principal pathway between the amygdala and the preoptic area of the hypothalamus, resulted in precocious ovarian stimulation. Conversely, stimulation of the amygdala in the young female rat was reported[25] to delay the onset of puberty. According to a recent report, androgen-induced precocious puberty in female rats is also inhibited by lesions in the ventrolateral hippocampus (adjacent to the medial amygdala)[26].

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Localization of Choline Acetyltransferase

The techniques for the histochemical detection of choline acetyltransferase, outlined in the following articles, will facilitate studies of the intracellular organization of cholinergic neurones and their distribution in the CNS.

Histochemistry at the Light Microscope Level

THERE has long been a need for a histochemical method for locating the enzyme choline acetyltransferase (acetyl-CoA : choline *O*-acetyltransferase, EC 2.3.1.6). It would provide valuable information on two difficult questions in particular. The first concerns the intracellular location of the enzyme and what position it occupies at the endings of cholinergic neurones. A reliable histochemical method should settle this and also help to resolve the problem of the intracellular transport of acetylcholine (ACh), from sites where it is synthesized to those—presumably the synaptic vesicles—where it is stored[1]. The second question is how to establish with certainty which central neurones are cholinergic. At present we depend largely on the histochemistry of acetylcholinesterase (EC 3.1.1.7) to determine which central pathways are cholinergic; but the distribution of this enzyme does not run parallel in all parts of the CNS with that of ACh, and its presence in high concentrations in tissues such as the cerebellum may have some other significance. On the contrary, from all that is known of the distribution of choline acetyltransferase it can be expected to give a surer indication of the distribution of ACh and hence of cholinergic neurones[2].

We have tested one possible histochemical method originally proposed by Burt[3], in modified conditions[4]. Thin sections of tissue are incubated in the presence of acetyl-CoA (ACoA), choline and a lead salt. Because acetylcholine is formed by the catalytic action of choline acetyltransferase, free CoA—which contains a sulphydryl group—is released and precipitated in the vicinity of the enzyme by the lead present in the solution. The precipitate so formed can then be converted to lead sulphide, which is readily seen under the microscope, by treatment with sodium sulphide (*p*H 7·5). As controls one or both substrates are omitted from the incubation mixture.

Many other reactions could, of course, lead to the release of free CoA from ACoA and it is therefore important to show that the formation of the histochemical end-product is choline-dependent. This was evidently not the case in Burt's experiments, for he reported that the reaction rate was only halved when choline was omitted from the medium: he suggested that this represented hydrolysis of ACoA by choline acetyltransferase in the absence of choline.

An alternative, or additional, possibility is that some hydrolysis of ACoA was catalysed by the acetylcholinesterase present in the tissue, for he did not include an anticholinesterase in his substrate mixture. We have found that a commercial preparation of bovine erythrocyte acetylcholinesterase readily hydrolyses ACoA (measured as described by Chase[5]), and that this can be prevented by eserine. In our modified system[4], the tissue sections (of rat spinal cord and spinal ganglia) were therefore pre-incubated with eserine, in a concentration of approximately 10^{-4} M, and eserine was also added to the substrate mixture which contained the following (in mM): eserine sulphate, 0·13; cacodylate buffer (*p*H 5·9), 33·0; ^{14}C-acetyl-CoA, 0·67; choline chloride, 10·0; and lead nitrate, 0·1. Radioactive ACoA was used so that the amount of ACh which formed in the incubation medium could be monitored.

The results of tests using this system showed that on average eserine considerably reduced the rate of reaction, and the degree of staining, in the absence of choline. The effect was variable, however, and in some sections of spinal cord fairly marked positive staining was obtained after incubation without added choline. Moreover, some cells from other parts of the nervous system, which according to biochemical measurements should contain little or no choline acetyltransferase, unexpectedly gave a positive reaction. For example, the Purkinje cell bodies in the cerebellum were regularly positive, and, somewhat more variably, some or most of the spinal ganglion cells became stained to some extent.

Clearly some staining occurred, or could occur, independently of the catalytic action either of choline acetyltransferase or of acetylcholinesterase and we decided to follow the rate of hydrolysis of ACoA when it was incubated with rat brain homogenate and with no added choline at 39° C. In these conditions there was a steady loss of ACoA, which did not occur if ACoA was incubated without the homogenate. The rate of loss of ACoA, which was found to be associated with the release of free acetate, was considerably depressed by 10^{-4} M eserine, but the rate of breakdown of ACoA was still substantial. Ascribing this to the action of esterases other than the cholinesterases, we investigated the effect of increased concentrations of eserine (up to 10^{-2} M) and of sodium fluoride (up to 3×10^{-2} M) added with or without eserine, both of which should inhibit some of the normally eserine-resistant esterases. Neither in fact had any significant effect on the rate of hydrolysis beyond what was achieved by 10^{-4} M eserine.

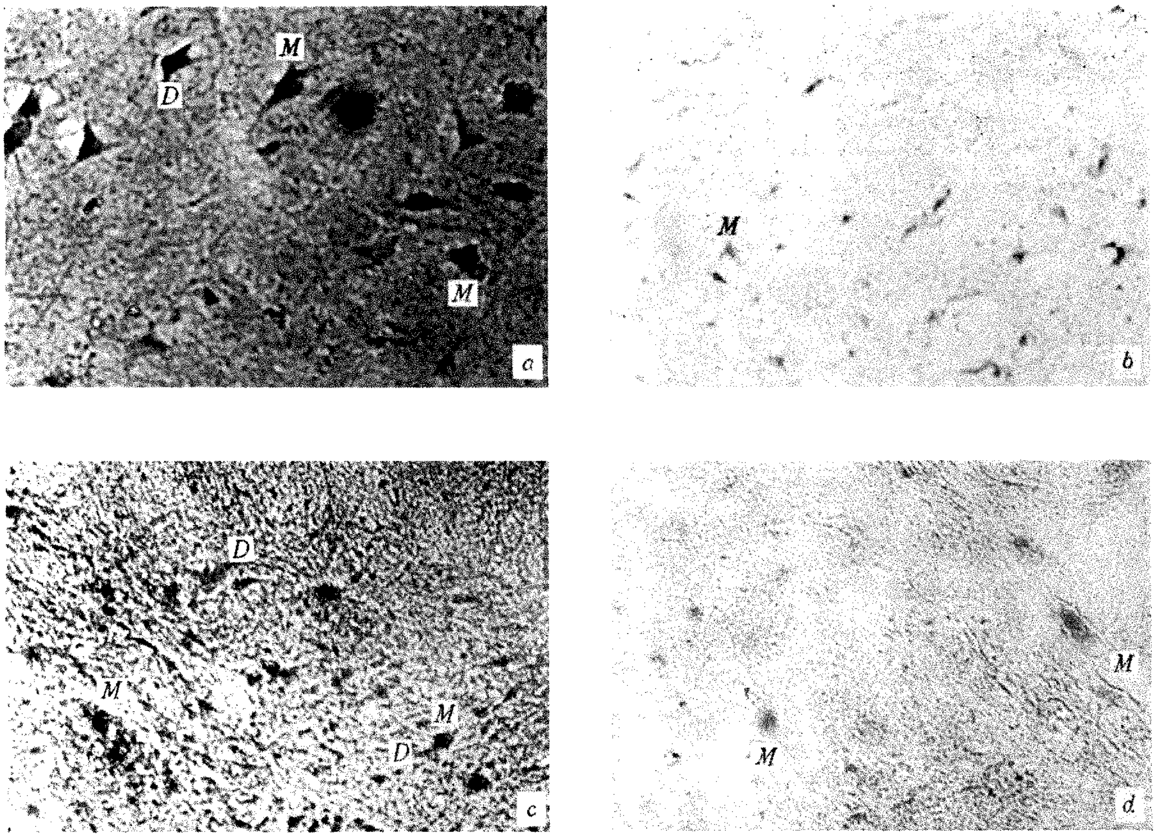

Fig. 1. Sections of rat spinal cord. *a* and *c*, incubated in the presence of DFP, ACoA, choline and lead; *b* and *d*, choline omitted. *a* and *b* were unfixed and incubated 4 h; *c* and *d* were fixed in 1 per cent formaldehyde before being incubated for 5 h. All sections were pre-incubated with DFP (10^{-3} M) for 30 min. *D*, Dendrite; *M*, motoneurone. ×240.

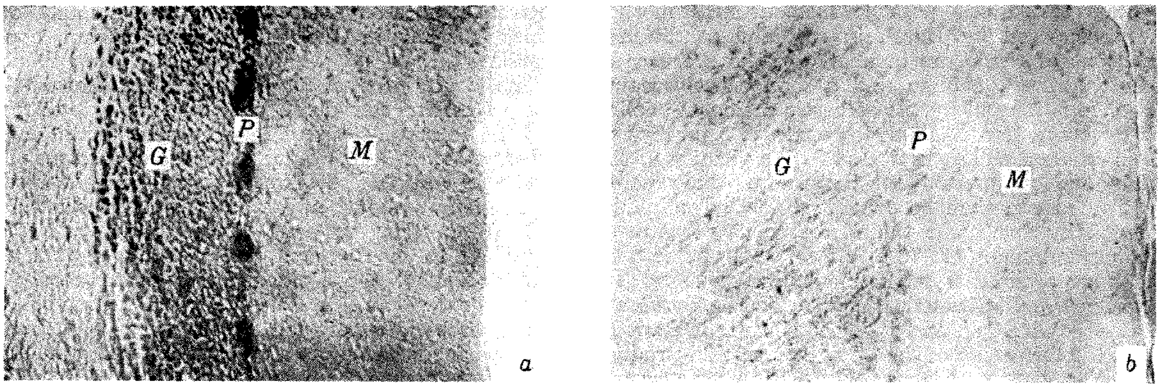

Fig. 2. Sections of rat cerebellar cortex, fixed with formaldehyde. *a*, Incubated with eserine, ACoA, choline and lead. There is marked staining of the Purkinje cells (*P*) and some of the granular (*G*) and molecular (*M*) layers. Incubation time, 5 h. *b*, Incubated in the presence of DFP, ACoA, choline and lead. Note that the Purkinje cells are not stained but slight activity remains in the granular (*G*) and molecular (*M*) layers. Incubation time 5 h. ×240.

We next examined di-isopropylfluorophosphate (DFP). Like eserine, in the concentrations which ensured the complete inhibition of acetylcholinesterase (10^{-5} to 10^{-6} M) and applied in the same way (including preincubation without the substrate), DFP slowed the rate of hydrolysis of ACoA considerably. More important, when the concentration of DFP was increased it slowed the reaction even more, until at a maximally inhibitory concentration of 10^{-3} M it reduced the rate of hydrolysis by 90 per cent. In view of its effectiveness DFP was then tested in the histochemical procedure. The results were much more clear-cut than with eserine. First, DFP as an inhibitor of the non-specific hydrolysis of ACoA did not interfere with the positive reaction of the ventral horn and other cells containing choline acetyltransferase; while by contrast with the earlier results, the omission of choline was associated with a very considerable reduction in their staining (Fig. 1). Thus the reaction produced in the presence of choline was not likely to have been obscured, or significantly added to, by other uncontrolled reactions, and was therefore probably to be attributed to choline acetyltransferase. Other biochemical tests established that 10^{-3} M DFP had no inhibitory effect on choline acetyltransferase.

Further evidence for the identification of the observed histochemical end-product with the catalytic action of choline acetyltransferase is that with the substitution of DFP for eserine in the incubation mixture, spinal ganglion cells and the Purkinje cells no longer reacted positively (Fig. 2). On the other hand, a number of cells in the red nucleus which may be expected to be cholinergic did continue to react.

How generally applicable and reliable the method will be depends on more extensive tests. It is desirable to fix tissue sections before they are incubated[6], for diffusion of enzyme from fresh frozen sections may lead to its false localization or to widespread deposition of the histochemical end-product. In the case of choline acetyltransferase it is difficult to achieve satisfactory fixation of the tissue without reducing the activity of the enzyme very seriously. Although a positive reaction can be obtained on formalin-fixed tissue (Fig. 1*c* and *d*), this was only achieved with a loss in the sensitivity of the reaction, and this problem has to be overcome. One precaution is to keep the ionic strength as low as possible because choline acetyltransferase is more soluble at high ionic strengths[7]. But this, too, has meant some loss of enzyme activity. Further, the inclusion of lead in the incubation medium and its adjustment at *p*H 5·9 both reduce the enzymic synthesis of ACh appreciably, and this may well mean that the method even when applied to unfixed tissue will not detect choline acetyltransferase at low concentrations. The concentration in nervous tissue is probably much lower than that of acetylcholinesterase, so it is not to be expected that the staining reaction of a given tissue for choline acetyltransferase will be as strong as for acetylcholinesterase even in optimal conditions.

Although we are developing the method for use with the light microscope, it is only at the electron microscopic level that questions about the intracellular location of the enzyme can be resolved. We tentatively suggest that the enzyme may be associated in the cell body with the ribosomes, in the axons with the neurofilaments, and in the nerve endings with some intervesicular material.

Note added in proof. Professor A. M. Burt has recently sent us a copy of an article (*J. Histochem. Cytochem.* (in the press)) describing in greater detail the histochemical procedure of ref. 3. Although his method relies on precipitation of the lead mercaptide of CoA to mark the sites of ACh synthesis, it differs in many important respects from our own. We should point out, however, that in his final procedure, Burt has found it useful to add an anticholinesterase, phospholine iodide.

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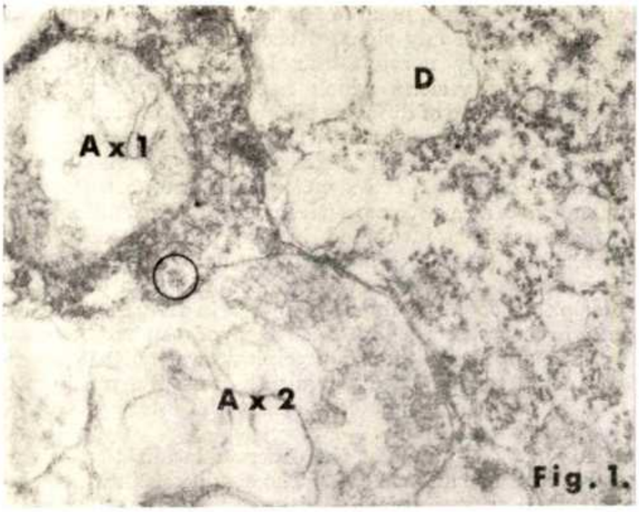
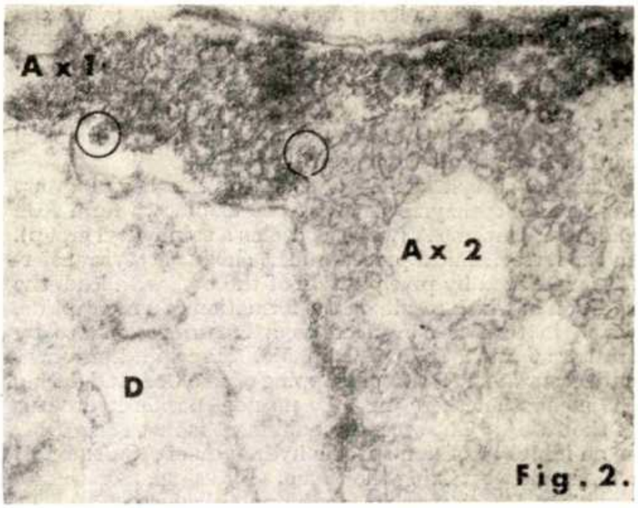
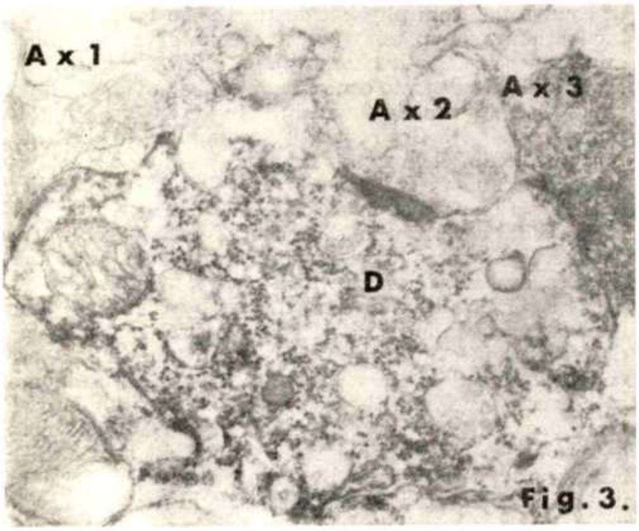
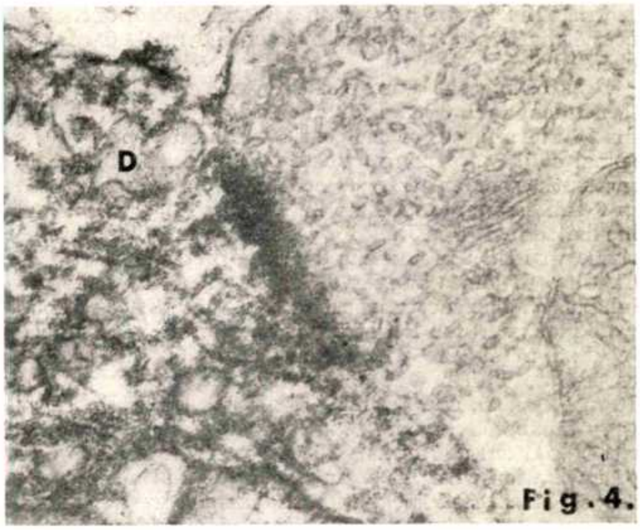
Ultrastructural Localization in Spinal Neurones

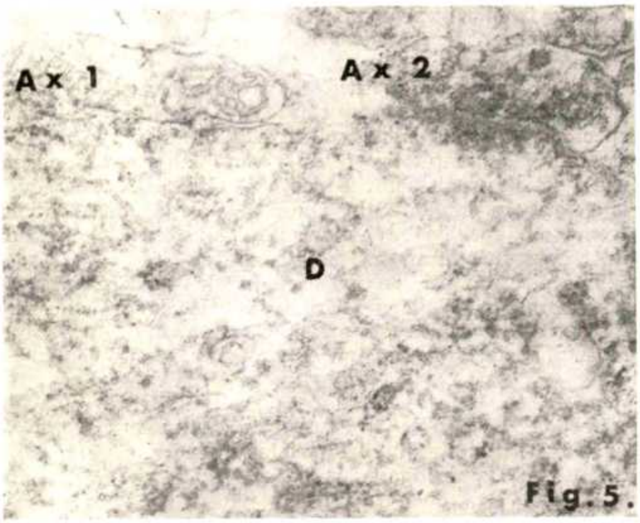
ACETYLCHOLINE (ACh), a chemical substrate for synaptic transmission, is synthesized by choline acetyltransferase, which is present in all cholinergic neurones (and is found as well in some non-nervous tissues[1]). It is detectable in the axon and its terminals and is thought to be in the cell body and dendrites too, but this has never been formally demonstrated although there is evidence that choline acetyltransferase, like most other neuronal proteins, is synthesized in the cell body and transported continuously at a low rate to the axon endings[2,3]. We have described a specific technique, based on that of Burt[4], for the localization of choline acetyltransferase at the light micro-











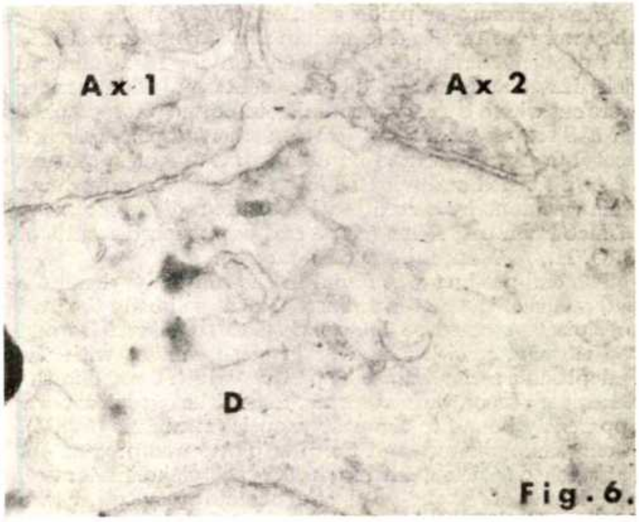


Fig. 1. Electron micrograph of the rat spinal cord. Choline acetyltransferase activity is present in the dendrite (D) and within an axon terminal (Ax 1). At the bottom left is another nerve terminal (Ax 2) which does not exhibit any reaction product. It is not possible to say whether in those vesicles marked by circles here and in Fig. 2 the precipitate is inside or on their surface. The swollen condition of the mitochondria shown in this and other figures results from incubation of the tissue in a hypotonic medium. × 39,000.

Fig. 2. Choline acetyltransferase positive (Ax 1) and negative (Ax 2) nerve terminals in close contact with a choline acetyltransferase negative dendrite (D). Note the reaction product on the outer surface of the synaptic vesicles. For explanation of circles see Fig. 1. Rat spinal cord. × 54,000.

Fig. 3. Much of the reaction product which has formed in a dendrite (D) is attached to the ribosomes and some larger vesicles. On the dendrite three different nerve terminals make synaptic contacts: of two in which there is no reaction product, one (Ax 1) contains a mixed population of synaptic vesicles, the other (Ax 2) contains ovoid vesicles; the third (Ax 3) is choline acetyltransferase positive and possesses round shaped vesicles. Rat spinal cord. × 26,000.

Fig. 4. A choline acetyltransferase positive dendrite (D) synapsing with an enzyme-negative terminal that has ovoid or elongated vesicles. Rat spinal cord. × 41,500.

Fig. 5. Positive dendrite (D) in synaptic contact with a choline acetyltransferase negative terminal (Ax 1) and a positive terminal (Ax 2). Pigeon spinal cord. × 32,200.

Fig. 6. Section of pigeon spinal cord incubated in the presence of ClACh perchlorate to inhibit choline acetyltransferase activity. × 20,400.

scope level[5,6], but to learn more of its position in the cell we need to be able to examine the ultrastructural localization of the enzyme under the electron microscope. To do this we have had to introduce several further modifications of the original technique, and we now report the procedure and illustrate it with results from the spinal cord of the rat and the pigeon.

Choline acetyltransferase transfers the acetyl group from acetyl-coenzyme A (ACoA) to choline to form ACh and free coenzyme A (which contains a sulphydryl group). In the histochemical procedure the choline acetyltransferase is localized by precipitation of the CoA with lead, the addition of which results in the formation of a mercaptide, which in turn is converted to a brown precipitate by treatment with sodium sulphide. Precipitation must only occur as the result of trapping of free CoA and during the incubation there must be no non-specific hydrolysis of the ACoA. The second requirement is met using DFP as an inhibitor of non-specific hydrolysis of ACoA (ref. 6) and the first by fixing the tissue. By using chloroacetylcholine (ClACh) perchlorate (unpublished results of D. Morris, D. S. Grewaal and P. K.) as an inhibitor of choline acetyltransferase, the specificity of the method can be checked.

Rats or pigeons were perfused with 1 or 2 per cent formaldehyde in 0·1 M sodium cacodylate buffer (pH 7·5) for 10 min; pieces of the cervical or lumbar part of the spinal cord were then removed and immersed for 30–60 min in the same fixative; after this the tissue was washed in 0·1 M sodium cacodylate buffer (pH 7·0) for 3 h, and incubated in 10^{-3} M DFP for a further 1 h. Some of the tissue was then preincubated in the presence of 10 mM ClACh perchlorate at pH 7·0 for an additional 30 min to inhibit choline acetyltransferase itself. The samples were incubated for 1 h at 25° C and 3 h at 37° C in the incubation mixture which consisted of 33 mM sodium cacodylate buffer (pH 5·9), 0·67 mM ^{14}C-acetyl-CoA, 10 mM choline, and 0·1 mM $Pb(NO_3)_2$. Control samples were incubated in a similar incubation mixture which either contained 10 mM ClACh perchlorate, following preincubation with the same reagent, or did not contain any choline. After incubation the samples were rinsed in sodium acetate buffer (pH 7·0) and transferred to a buffered sodium sulphide solution (pH 7·5) for 5 min; they were then washed in sodium acetate buffer, postfixed in 1 per cent osmium tetroxide solution[7] for 15–20 min and dehydrated and embedded in the usual way. Thin sections were cut with a Reichert ultramicrotome and photographed on a Siemens 'Elmiskop' electron microscope. Labelled acetyl-CoA was used to be able to measure the amount of ACh formed and released into the incubation medium. This was done by a modification of the radiometric assay of McCaman and Hunt (ref. 8 and unpublished results of C. O. H., A. Maneckjee and D. Morris).

The end-product was found in the cell body, dendrites and axons of the motoneurones. In the cell body the lead precipitate seemed to be attached to some vesicles and the ribosomes, either free or in close contact with the endoplasmic reticulum. The product which formed in the dendrites was on the ribosomes, free in the cytoplasm and apparently bound to the postsynaptic membrane (Fig. 4). Along the axons most of the precipitate was present on, or attached to, the filaments; unlike acetylcholinesterase[9], no evidence of the end-product of choline acetyltransferase was seen in the tubules. In some of the axon terminals (Figs. 1, 2, 3 and 5) evidence of relatively concentrated enzyme activity can be seen, corresponding presumably to cholinergic nerve terminals. The reaction end-product appeared on and between the synaptic vesicles and on the filaments; none could be seen in the mitochondria and very little on the inner surface of the axolemma.

A point of particular interest is that the reaction end-product was evident only in axon terminals which contained round—as distinct from ovoid—shaped vesicles. Not all axons with round vesicles displayed a positive reaction; on the other hand, none of those with ovoid or elliptical vesicles showed any signs of the product. There were, however, many nerve terminals containing predominantly round vesicles which had no enzyme activity. Possibly because of diffusion of the enzyme during incubation, or at an earlier stage, some reaction product was found extracellularly between membranes where one or more belonged to a cell structure, dendrite, cell body or axon, which also gave a positive reaction (Figs. 2, 4 and 5).

Most samples incubated in the presence of ClACh perchlorate (Fig. 6) or in the absence of choline displayed no evidence of enzyme activity. In some, however, lead precipitate could be found in places in the glia cells, on and in between the outer surface of the membranes. But the appearance of this precipitate was quite different and could readily be distinguished from the precipitate seen in samples incubated without the inhibitor and in the presence of choline.

To what extent might the observed localization of the end-product be an artefact ? The conditions in which the enzyme is operating in the histochemical procedure are, of course, far from physiological: the solution used for incubation was hypotonic (which accounts for the swollen appearance of the mitochondria), its pH was considerably lower and its substrate concentration higher than normal, while the salts usually found in living tissue were hardly represented in it. Such conditions could lead to abnormal precipitation of the enzyme and we therefore cannot yet attach too much significance to the detailed localization of the reaction product in the cell. We attach more significance, however, to the evidence of enzyme occurring exclusively in those nerve terminals which contain round vesicles, and not in those which have elongated or ovoid vesicles. It has been suggested, first by Uchizono[10] and later for the spinal cord by Bodian[11], that morphological differences in the synaptic vesicles characteristic of nerve endings may reflect differences in the nature of the chemical transmitter which they contain. Our results fit with this view and suggest that nerve terminals with elongated vesicles are not cholinergic; but that those which contain rounded vesicles may be either cholinergic or non-cholinergic. If it is assumed that in non-cholinergic neurones the elongated vesicles contain a different neurohumour from that present in the round ones, then at least three different transmitter substances may act on the same cell body or dendrite.

In the preceding article[6], we emphasized choline dependence as a necessary condition to be fulfilled if the specificity of the histochemical reaction is to be demonstrated. We emphasize equally that the appearance of the reaction product in the electron microscopy technique is effectively prevented by the use of an inhibitor which seems to be specific for choline acetyltransferase when used in the presence of an anticholinesterase.

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Molecular Mechanics of Translation: a Reciprocating Ratchet Mechanism

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A model is suggested for protein synthesis which depends upon conformational changes in tRNA and allosteric transitions in place of translocation.

CURRENT understanding of the nature of translation is embodied in the A-site–P-site model of the ribosome. The A site holds the incoming aminoacyl tRNA, the P site the peptidyl tRNA. As a consequence of peptidyl transfer the former tRNA is "translocated" from A site to P site[1,2]. In the past decade much evidence has accumulated consistent with this model, but it remains unproven[2-12]. This is not important, however, for the model in any case is too vague to be of real theoretical value. Clearly, what is needed is a conception of translation specific enough to suggest experiments that will elucidate the molecular mechanics of the process. This article is intended to fulfil this need.

It seems quite impossible to avoid invoking tRNA-like entities (that is, an "adapter" system) as an integral part of translation from its very inception—which is sufficient reason for suspecting the basic molecular mechanics of the process to lie in the properties of this molecular species[13-16]. For this reason it may be of value to examine the anticodon arm of tRNA—that portion of the molecule apparently most directly involved in translation. A structure for this arm has been suggested by Fuller and Hodgson[17]. Because the arm always comprises seventeen nucleotides, the first and last five of which complement one another, it should possess a double helical "stalk" of five base pairs. Fuller and Hodgson maintain that in the most probable overall configuration for the anticodon arm total base stacking is optimized. Sterically this could mean that one of the chains of the double helical stalk continues for five additional residues in a single stranded extension with the same pitch and axis as the stalk proper[17]. Extending either chain in this fashion produces a similar configuration, in which the three anticodon bases reside at the top of the extension. The two possibilities differ, however, in that: (1) extension of the 3′–5′ chain of the stalk causes two purines to underlie the anticodon bases, whereas extension of the 5′–3′ chain causes two pyrimidines to do so; and (2) in the first instance the III* anticodon base is topmost, but in the second it is the I* anticodon base[17]. (These two conformations of the anticodon arm will be referred to as "*FH*" (3′–5′ chain extended) and "*hf*" (5′–3′ chain extended).) The stacking properties of purines, Fuller and Hodgson argue, would cause the *FH* conformation to be energetically favoured*.

Conformational Changes in tRNA

While the argument that total base stacking interactions must be optimized seems reasonable, it should nevertheless be applied not to tRNA (or the anticodon arm thereof) in isolation, but rather to tRNA in the act of translation. In this case one has to consider in addition to the *FH*-type of structure another formation, the duplex of six base pairs potentially formed between the "peptidyl" and the "aminoacyl" codons and their respective anticodons. This sextuplet duplex structure (*SDS*) is compatible with the peptidyl–anticodon, but not the aminoacyl–anticodon arm being in the *FH* conformation. However, were the aminoacyl–anticodon arm to assume the *hf* conformation, a combined *FH–SDS–hf* helix could form. As Fig. 1 shows, these three overlapping helical structures are coaxial, and the "translation complex" they form has an overall length equivalent to twenty base pairs. Since total base stacking energy in such a complex seems greater than would be the total base stacking energy were both the peptidyl and the

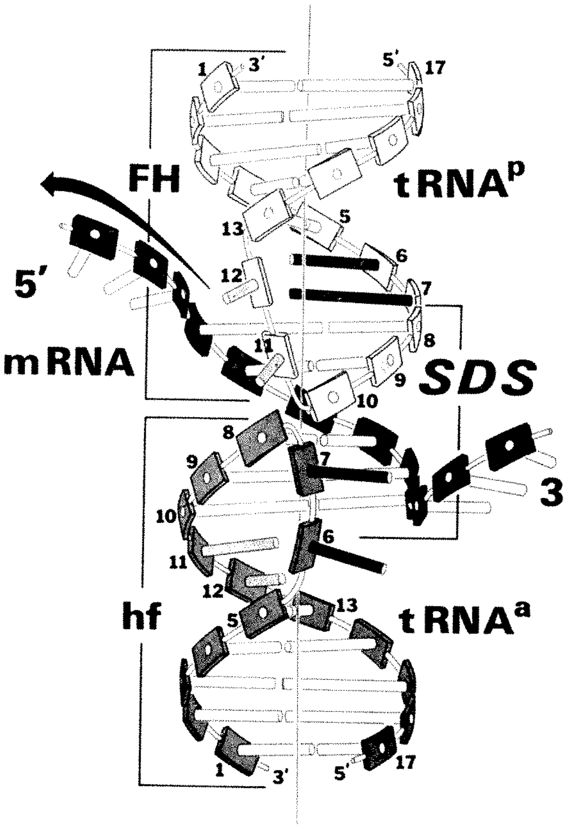

Fig. 1. The basic translation complex. The complex (in essence an RNA double helix the equivalent of twenty base pairs in total length) comprises anticodon arms of both a peptidyl tRNA (tRNA^p) in the *FH* conformation and an aminoacyl tRNA (tRNA^a) in the *hf* conformation, plus their respective codons (combined with the anticodons proper in a central "sextuplet duplex structure" or *SDS*). Bases Nos. 6 and 7 (solid bars) are not paired and are excluded from the helix proper in an *hf* conformation. Bases Nos. 11 and 12 (stippled bars) are similarly unpaired and are excluded from the helix in the *FH* conformation. Numbering of bases is that used in ref. 17. The 5′ and 3′ ends of all chains are shown. mRNA as drawn would move from right to left in translation (indicated by the arrow). The axis of two-fold rotational symmetry (dyad) relating the anticodon stalks is formed by the intersection of two planes; one is perpendicular to the axis of the complex at its midpoint; the other contains that axis and is perpendicular to the bars (base pairs) linking the No. 5–No. 13 positions in either anticodon stalk. See text for further details.

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* The base pair tilt in RNA double helices also places constraints on these structures, in that bases Nos. 11 and 12, excluded from the helix in the *FH* conformation, and Nos. 6 and 7, excluded from the helix in the *hf* conformation, must span different grooves of the structure. Nos. 11 and 12 span the "major groove" in an *FH* structure, Nos. 6 and 7 span the "minor groove" in an *hf* structure[17].

aminoacyl–anticodon arms in the *FH* conformation, strong support is provided for the idea that during translation the anticodon arm of tRNA undergoes a conformational change—an *hf* → *FH* transition.

A very simple mechanism for message RNA movement and the maintenance of reading frame is inherent in an *hf*→*FH* transition. When paired to an anticodon in the *hf* conformation, a codon lies in the helical strand defined by the 3′–5′ chain of the anticodon stalk (Fig. 1). In this configuration the I base of that codon is topmost, and the preceding codon can lie directly above it (with the same helical pitch and axis). When the *hf*→*FH* transition occurs, however, the codon in question then resides in the other strand of the double helix (that is, the one defined by the 5′–3′ chain of its anticodon stalk) and therefore is inverted, with its III base now topmost. Consequently the preceding codon can no longer share a common axis with this codon, but the succeeding codon now can. This inversion of the codon relative to its anticodon stalk provides a potential mechanism not only for mRNA movement but also for tRNA displacement and strict maintenance of reading frame.

This mechanism is in no way inconsistent with A-site–P-site theory, although it restricts the possible orientations of the two sites, that is, the P site must include the top half of the translation complex as drawn in Fig. 1, the A site its bottom half. These considerations do, however, suggest an entirely different, simpler alternative for the molecular mechanics of translation.

Translation as an Allosteric Phenomenon

"Translocation" of a (more or less static) tRNA molecule from one site to a different kind of site is the essence of the A-site–P-site model. The alternative is an allosteric type of mechanism, in which an essentially antisymmetric dimer undergoes cyclic transitions between two functionally equivalent states—that is, $A_1{\cdot}A_2' \rightleftarrows A_2{\cdot}A_1'$—and no translocations occur. (The two functionally equivalent subunits, A and A′, each contain a complex of a tRNA molecule, its codon, and a corresponding ribosomal site. The subscripts, 1 and 2, designate the two possible states of a subunit.) The simplest specific version of this allosteric mechanism is illustrated in Fig. 2.

In the figure, S and S′ signify the functionally equivalent ribosomal sites, each containing one tRNA molecule bound to its codon. The sites are situated so that the two tRNAs can interact to form the translation complex of Fig. 1. Fig. 2*A* (the $A_1{\cdot}A_2'$ state) depicts a point in the middle of a translation, for example, with $tRNA_3$ (peptidyl, *FH* conformation) in the S site and the next tRNA, $tRNA_4$ (aminoacyl, *hf* conformation) in the S′ site. As a consequence of peptidyl transfer, $tRNA_4$ will (for reasons not specified) undergo an *hf*→*FH* transition; see Fig. 2*B*. $tRNA_4$ does not leave the S′ site in the process, and its anticodon stalk is here pictured to remain completely stationary while the duplex composed of codon No. 4 and its anticodon undergoes a "flip" (as a single unit)*. The "flip" necessarily breaks the original *SDS* helical segment and displaces codon No. 3 from the axis of the translation complex, causing $tRNA_3$ to vacate site S as a result. The same event, moreover, pulls the mRNA so that codon No. 5 now enters the S site. Fig. 2*C*, where $tRNA_5$ (aminoacyl, *hf* conformation) has also entered the S site to pair with codon No. 5, then corresponds to the $A_2{\cdot}A_1'$ state. At the next peptidyl transfer event $tRNA_5$, without moving its stalk, shifts into the *FH* conformation, displacing both $tRNA_4$ and its codon from site S′, and bringing codon No. 6 into that site (Fig. 2*D*). When $tRNA_6$ (aminoacyl, *hf* conformation) enters site S′, the cycle is completed (that is, the translation complex again has the $A_1{\cdot}A_2'$ form of Fig. 2*A*).

In terms of these numberings, even numbered tRNAs are "decoded" in one site, odd numbered ones in the other. This allosteric transition mechanism pulls the mRNA tape through the ribosome in a manner reminiscent of a double reciprocating ratchet. Because conformational changes in the tRNA molecule are primarily responsible for the mechanics involved, the main roles for the ribosome may be in providing the static sites, S and S′, in catalysing the actual peptidyl transfer, and perhaps in assisting somehow the *hf*→*FH* transitions. It should be stressed that this version is a "first approximation" model, and that one can anticipate refinements and/or modifications of non-essential features, if the basic mechanics prove to be essentially correct. For example, entry and/or exit sites, preceding or succeeding (respectively) the actual translation complex might be contemplated for any of a number of reasons.

Symmetry of Ribosomal Sites

Most of the arguments that gave rise to and support the A-site–P-site theory come from experiments demonstrating properties unique to one or the other of the ribosomal subunits. For example, codon-specific tRNA binding is a property of the 30S subunit, while the 50S subunit seems to hold the tRNA carrying the nascent peptide and to possess factors involved in peptidyl transfer[3,6,9]. The essence of the A site seems to be in the 30S subunit, while the P site resides in its 50S counterpart. Were any site to comprise parts from both the 30S and the 50S subunits, however, results such as these might well be expected. (There is no *a priori* reason for an mRNA or a tRNA molecule to react in the same fashion with both parts of any given site; nor is it necessary for the aminoacylated form of tRNA to react with any site—or part thereof—in the same way that its peptidylated counterpart does.) Evidence such as I have discussed therefore can neither prove the A-site–P-site theory, nor rule out the allosteric model alternative.

I do not intend to discuss in detail the relationship of this model to the overall tertiary structure of tRNA or to the structure of the ribosome. A few obvious points must be made, however. It is reasonable to expect each of the postulated S sites to comprise parts from both the 50S and the 30S ribosomes, so that the axis of two-fold rotational symmetry (that is, the dyad described in the caption of Fig. 1) passes through and applies to both ribosomal subunits. Evidence at present ostensibly does not favour such a symmetrical ribosome; for example, neither ribosomal subunit contains more than one copy of any given type of protein[18]. It must be realized, however, that all we require here is functional (not primary structural) symmetry, that a pseudo-symmetry may be all that is actually needed, and that symmetry considerations could apply only to a portion of each ribosomal subunit in any case.

On the other hand, the 50S particle has always seemed likely to be an "evolutionary dimer" by its overall molecular weight, the molecular weight of its RNA, and the number of protein molecules it contains (all of which are double their counterparts in the 30S particle)[18]. More recently it has been found that all of the (T1 RNase) oligonucleotide segments in the 23S rRNA that contain post-transcriptionally modified bases are present in two copies per molecule, as are some segments of this type from the 16S rRNA—consistent with, and suggestive of, the required symmetry[19]. Certain nucleic acid hybridization measurements also suggest some sort of two-fold symmetry to the primary structure of the 23S rRNA[20].

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* This "flip" can be simulated using a beer can as follows: the right hand grasps the upright can. The fingers, simulating the position of the codon, should be not quite horizontal but tilted slightly upward. The hand then rotates as a right-handed screw, the wrist simultaneously turning so that the can's axis becomes horizontal with its "top" facing to the left. Then the hand rotates in the reverse direction, the wrist now turning so that the can's axis is once again vertical, but this time with the can inverted. The two groups of bases underlying the codon–anticodon unit (that is, Nos. 11 and 12 on the one strand, Nos. 6 and 7 on the other) each rotate approximately clockwise (viewed from the anticodon stalk) during the "flip", causing the pyrimidine group (Nos. 11 and 12) to swing out of, the other group (Nos. 6 and 7) to swing into, helical pitch.

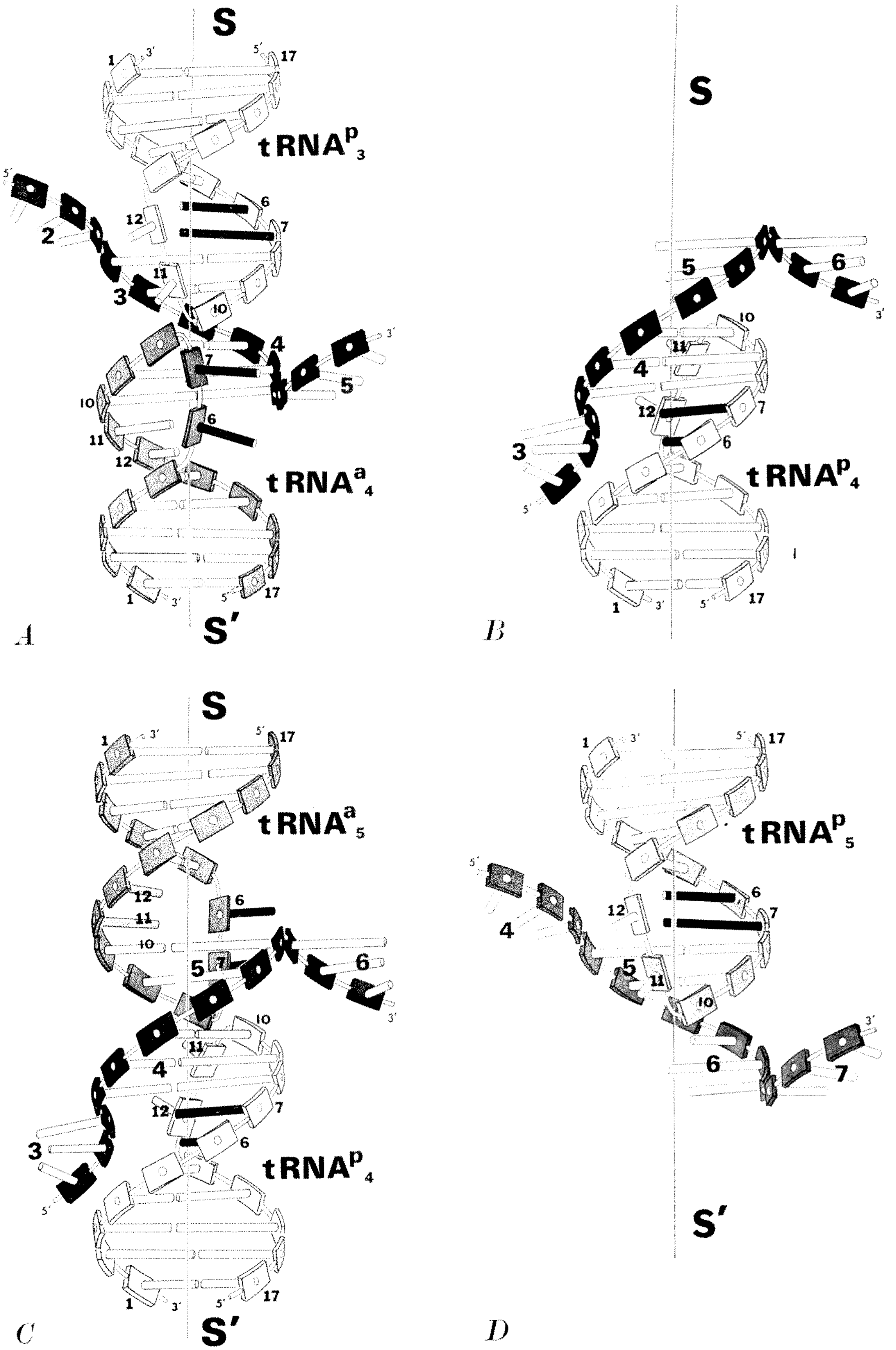


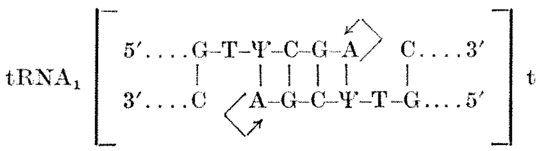
Fig. 2. The molecular mechanics of translation. *A*, The basic translation complex of Fig. 1, with the peptidyl tRNA (superscript "p"), in *FH* conformation, in the S (upper) site and the incoming aminoacyl tRNA (superscript "a"), in *hf* conformation, in the S′ (lower) site. Subscripts designate the time order of appearance of tRNAs in translation. Large numbers designate codons in their order of occurrence in mRNA; other numbering is as in Fig. 1. The $hf \rightarrow FH$ conformational transitions and consequent mRNA movement, occurring in *B*, *C* and *D*, are explained in the text.

Tertiary Structure of tRNA

Although the multiple conformations of a tRNA molecule demanded by the present model have yet to be identified, it should be noted: (*a*) that "denatured" (as opposed to "normal") forms of various tRNAs have been reported, and (*b*) that uncharged tRNAs exist in what appears to be the *hf* conformation[21-23].

In any simple form this model requires that the overall structures of the peptidyl and the aminoacyl tRNAs (in a translation complex) be such that the incoming amino-acid and the nascent peptide are brought into approximate juxtaposition. If the gross tertiary structures of these two tRNAs are taken to be approximately the same, then the two CCA ends should meet in the vicinity of the symmetry axis. Of the models so far proposed for tRNA tertiary structure, only two seem to fulfil this requirement (the model attributed to Doctor *et al.* and that proposed by Melcher[24-26]).

The model discussed here also suggests that tRNA may pass through a set of functionally interrelated configurations during translation, of which the *hf*→*FH* is only a part. In view of the variety of tRNA tertiary structures that seem to be sterically feasible, larger changes—for example, rotation of an arm relative to the rest of the structure—might be considered[26]. Particular attention should be given to the "TΨC" arm, common to all tRNAs. In its general form it is identical to any anticodon arm, immediately suggesting the possibility of *hf*↔*FH* transitions. It should further be noted that in the *hf* conformation one such common arm can generally form a coaxial double helical segment with another common arm, a structure that includes the following symmetrical base paired segment:



The allosteric translation model is too crudely delineated at present to warrant a detailed discussion of the exact roles it would assign to the various initiation, termination, binding and so on, factors required for protein synthesis[2,10,11,27]. In that the present model (in any simple form) specifically does not utilize "translocations" (moving of the tRNA-codon complex from one translation site to another), however, we must briefly consider the so-called "G", or "translocase", factor customarily assigned the role herein proscribed[2]. Clearly, all its reported characteristics[2] are consistent with a role in which "translocase" facilitates transitions—*hf*→*FH* or others.

Although it has obvious relevance to cellular dynamics, I feel the ultimate value of the model proposed herein will lie in its evolutionary implications—that is, it places the burden for translation in any primitive system on the tRNA–mRNA interaction, and so allows the "protoribosome", at least in principle, to be a rather simple and static entity. Detailed evolutionary implications of this model are presented elsewhere (my work, in preparation).

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Control of Translation by T4 Phage: Altered Binding of Disfavoured Messengers

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A messenger selection process operates in infected cell extracts at the level of polypeptide chain initiation; and the agent responsible is present chiefly in the factor fraction of the ribosome.

After infection of *Escherichia coli* by T-even phages, host protein synthesis is switched off[1-4], although the overall rate of protein synthesis continues almost unabated[5,6]. This change may be mediated at the transcriptional level for T4 by synthesis of a new sigma factor[7,8] which alters the initiation specificity of the host RNA polymerase, thereby allowing preferential synthesis of phage-specific RNA. Whether there is control at the translational level is less well understood. From the findings that RNA phages are unable to replicate in cells superinfected with T4 (refs. 9–11), Hattman and Hofschneider[10] have suggested that T4 exerts its control by interfering with the capacity of viral RNA to function as messenger. More recently, Hsu and Weiss[12] and Schedl, Singer and Conway[13] have reported that a factor from T4-infected cells induces an alteration in host ribosomes which restricts the translation of host[12] and other RNAs unrelated to T4 (refs. 12 and 13) but permits normal translation of

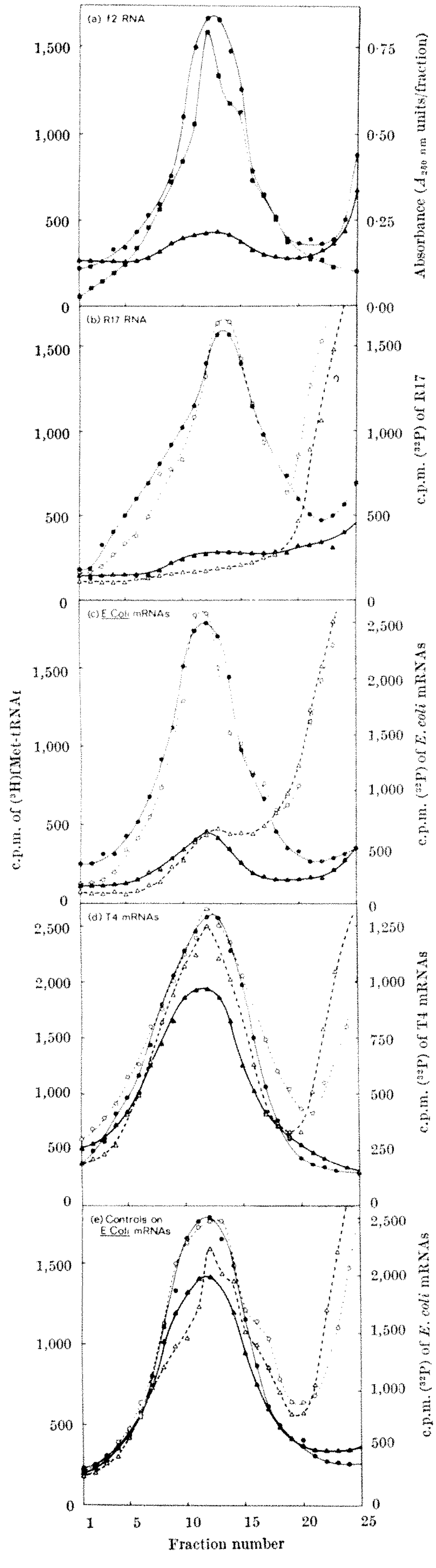

Fig. 1. Ribosomal binding on sucrose density gradients. For *a–d* binding of fMet-tRNA$_f$ and mRNAs was followed on gradients. Absorbance ($A_{260\ nm}$ units/fraction), ■—■; c.p.m. of ^{3}H-fMet-tRNA$_f$ with *E. coli* factors, ●—● or T4 factors, ▲—▲; and c.p.m. of ^{32}P-labelled messenger RNAs with *E. coli* factors, ○ - - - ○ or T4 factors, △ - - - △. For *e*, *E. coli* mRNAs were incubated with either *E. coli* factors (●, ○) or T4 factors (▲, △) in the complete reaction; the RNA was extracted and reincubated with the complete *E. coli* system before following the binding on sucrose density gradients. ^{32}P-labelled messenger RNAs from *E. coli* MRE600 and T4 infected cells were prepared at 37° C in 100 ml. cultures of 0·1 M Tris-HCl (*p*H 7·4), 0·02 M KCl; 0·09 M NaCl; 0·02 M NH$_4$Cl; 0·001 M MgSO$_4$; 0·4 per cent glucose; vitamin free casamino-acids and Difco bacto-peptone, both 2 g/l. (medium II). The exponentially growing cells were pulsed for 7 min with radioactive isotope, while the T4 infected cells were labelled from 5 until 12 min after infection. Isolation was as in Table 1, and the fractions corresponding to a 12S to 18S region were pooled for the messenger preparations. ^{32}P-labelled R17 RNA was a gift from Dr J. Nichols and Mr P. Jeppesen, isolated according to the method of Anderson *et al.*[31]. ^{32}P-labelled and non-radioactive messenger preparations were mixed in the following experiments. The reaction mixtures (50 µl.) contained the same buffer, GTP, and salt solutions as in Table 1, with 210 pmoles of *E coli* ribosomes, 100,100 c.p.m. of ^{3}H-labelled fMet-tRNA$_f$ (2,050 c.p.m. per pmole) and either 190 µg of *E. coli* factors (●—●), or 210 µg of factors from T4 infected cells (▲—▲). The messengers used were: *a*, f2 RNA, 3·3 $A_{260\ nm}$ units; *b*, R17 RNA, 2·0 units and 100,000 c.p.m. ^{32}P; *c*, host mRNAs, 3·1 units and 401,000 c.p.m.; *d*, T4 mRNAs, 2·6 units and 140,000 c.p.m. The procedure was as for Table 1, except that at the end of the incubation period the reactants were chilled on ice, 40 µl. of each mixture layered on a 5 ml. 5 to 20 per cent sucrose density gradient in 0·05 M Tris–HCl (*p*H 7·4); 0·05 M NH$_4$Cl; 0·005 M magnesium acetate (buffer B) and sedimented at 40,000 r.p.m. for 110 min in a Beckman model 'L' centrifuge with an 'SW50' rotor[13]. Two drop fractions (approximately 0·13 ml.) were collected from the bottom of the gradient directly into 2 ml. of 15 per cent trichloroacetic acid (TCA), and the precipitate was retained on nitrocellulose filters which were washed with 3 × 2 ml. addition of 5 per cent TCA, dried, and counted in a liquid scintillation spectrometer. "Spill-over" of ^{32}P c.p.m. into the ^{3}H channel of the spectrometer was less than 2 per cent. The c.p.m. of ^{3}H-labelled fMet-tRNA$_f$ (———, with closed symbols) and ^{32}P-labelled mRNAs (- - -, with open symbols) could be followed as a function of fraction number, whilst in *a* the total $A_{260\ nm}$ units of each fraction from the complete reaction mixture was recorded (■—■). For *e* host mRNAs were incubated in the same complete binding reaction as for *c* with either factors from T4 infected cells (▲—▲) or factors from host cells (●—●). On completion of the reaction, the mixtures were deproteinized by extraction with phenol and 2 per cent sodium dodecyl sulphate, and the RNA was precipitated with ethanol. 386,000 c.p.m. of this ^{32}P-RNA was then reincubated in the normal binding reaction with *E. coli* factors and ribosomes in both mixtures and the resultant ribosomal binding followed on sucrose density gradients.

T4 messenger RNAs[12]. (W. Salser and R. F. Gesteland, quoted in ref. 10, have also observed restricted translation of R17 RNA.)

We now demonstrate by binding experiments that a messenger selection process operates in infected cell extracts at the level of polypeptide chain initiation. The agent(s) responsible for this effect is present chiefly in the "factor"* fraction of the ribosome, which also contains several new proteins made after infection. The following article[14] shows that this "factor" interferes unequally with the initiation of protein synthesis at the three known genes of phage R17.

T4 Factors preclude Host mRNA Binding

Because the rate determining step in protein synthesis may be the formation of the initiation complex between fMet-tRNA$_f$, ribosomes, initiation factors, GTP and messengers[15–19], this is a probable point for translational control. We therefore tested the ability of host and T4 messengers to form initiation complexes with salt-washed ribosomes and factors, derived both from normal and from T4 infected cells. As messengers we also used the R17 and f2 RNAs. Because T4 excludes R17 or f2 phages, if ribosomes from infected cells display altered specificity for host mRNAs they may also do so for the viral RNAs.

Table 1 illustrates a striking difference in the ability of the RNAs to function as messengers as judged by the ribosomal binding of fMet-tRNA$_f$ on nitrocellulose filters[20]. T4 mRNAs are capable of forming a strong complex with all permutations of salt-washed ribosomes and factors, but R17, f2 and host messenger RNAs are unable to do so with either T4 washed ribosomes and T4 factors or *E. coli* ribosomes and T4 factors. Thus the complete ribosome from T4 infected cells is unable to form a stable initiation complex with host mRNAs, and this effect is localized largely in the factor fraction of

* The term "factor" denotes those proteins obtained from the supernatant after sedimenting ribosomes through a 2 M NH$_4$Cl buffer, and includes the known initiation factors. The prefix host or T4 designates whether the component has been isolated from *E. coli* or T4 infected *E. coli* cells.

Table 1. NITROCELLULOSE FILTER BINDING OF fMet-tRNA$_f$ TO RIBOSOMES WITH DIFFERENT MESSENGERS

E. coli ribosomes	T4 ribosomes	*E. coli* factors	T4 factors	*E. coli* mRNAs	T4 mRNAs	R17 RNA	f2 RNA	ApUpG
+	−	−	−	0·6	0·4	0·4	0·6	0·5
+	−	+	−	4·8	3·8	5·4	4·4	5·3
+	−	−	+	1·0	3·8	0·7	1·0	5·2
−	+	−	−	0·5	0·4	0·1	0·3	0·4
−	+	+	−	3·9	4·5	4·7	4·0	5·0
−	+	−	+	0·8	5·3	0·3	0·5	5·6

T4 phage infected cells were prepared as follows: 30 l. of *E. coli* MRE600 cells were grown at 37° C in 0·02 M Na_2HPO_4; 0·011 M KH_2PO_4; 0·047 M NaCl; 0·009 M NH_4Cl; 0·001 M $MgSO_4$; 0·4 per cent glucose Difco bactotryptone, 5 g/l. (medium I) to a density of 3×10^8 per ml., infected with T4 phage at a multiplicity of 10× phage to bacteria, superinfected at a multiplicity of 4× 7 min later, and finally collected 13 min after the first infection. Cell survival after 2 min was less than 0·5 per cent, and approximately 40 g wet weight of cells was collected by centrifugation and stored frozen. Uninfected *E. coli* MRE600 cells were obtained from the Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire. The ^{14}C-labelled pure fMet-tRNA$_f$ (specific activity 217), ApUpG, crude initiation factor preparation from a 2 M NH_4Cl buffer washing of ribosomes, and the high salt-washed ribosomes themselves were prepared as previously reported[27], except that the ribosomes were sedimented twice through the 2 M NH_4Cl buffer. All ribosomal components were aliquoted, and stored in liquid nitrogen before use. R17 RNA was prepared according to Gesteland and Boedtker[28]; f2 RNA was a gift from Mr M. Hooper prepared after the method of Webster *et al.*[29]. For the messenger RNAs from host and T4 infected cells the *E. coli* MRE600 cells were grown to a density of 3×10^8 per ml., fast cooled, collected by centrifugation, and immediately lysed with lysosyme—EDTA and 'Brij 35' (ref. 30). The RNA was deproteinized by phenol extraction, concentrated by ethanol precipitation and fractionated on sucrose density gradients, where fractions corresponding to maximum activity for initiation complex formation were pooled (manuscript in preparation) (approximately 12S to 18S for both T4 and host mRNAs). The initiation complex formation with these messengers depended on the presence of all components (that is, fMet-tRNA$_f$, ribosomes, GTP, and factors). Each reaction mixture (50 μl.) contained 0·1 M Tris-HCl (*p*H 7·2); 0·055 M NH_4Cl; 0·0055 M $MgCl_2$; 0·0002 M GTP; 11·4 pmoles fMet-tRNA$_f$; 34 pmoles of high salt-washed 70S ribosomes from either host or T4 infected cells, and 42 μg of crude factors from host or 40 μg from T4 infected cells where indicated. The messengers used were one of the following ($A_{260\ nm}$ units): *E. coli* mRNAs (1·2); T4 mRNAs (1·1); R17 RNA (1·4); f2 RNA (1·1); or ApUpG (0·12). All messengers were preincubated for 2 min at 37° C in 0·0001 M Na_2EDTA solution before addition of the remaining reactants, and incubated for a further 10 min at that temperature. The reaction was terminated by dilution sixty-fold with ice-cold 0·1 M Tris-HCl (*p*H 7·2); 0·05 M NH_4Cl; 0·005 M $MgCl_2$ (buffer A) followed by filtration through nitrocellulose filters. These filters were subsequently washed three times with 3 ml. of buffer A, dried, counted in a liquid scintillation spectrometer, and the pmoles of ^{14}C-fMet-tRNA$_f$ retained on the filter recorded.

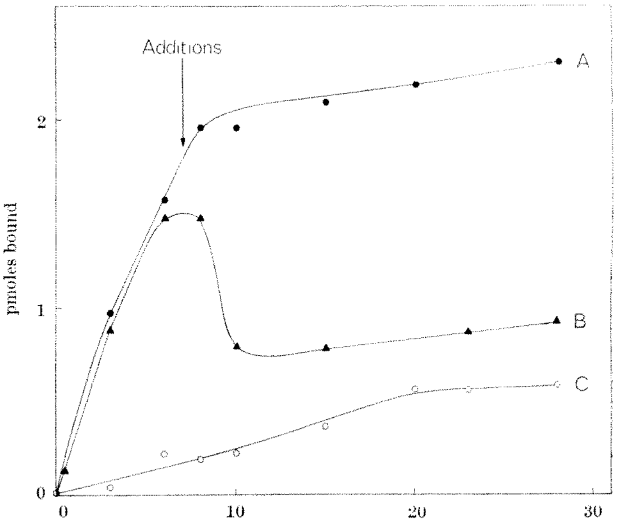

Fig. 2. Competition of host and T4 factors for initiation complex formation. *A*: host ribosomes + host factors; addition host factors. *B*: host ribosomes + host factors; addition T4 factors. *C*: host ribosomes + T4 factors; addition host factors. Reaction mixtures (500 μl.) contained the same buffer, GTP and salt solutions as in Table 1 with 340 pmoles *E. coli* ribosomes, 110 pmoles of ^{14}C-fMet-tRNA$_f$, 12·3 $A_{260\ nm}$ units of *E. coli* mRNAs, and either 420 μg of factors from host cells (*A* and *B*) or 400 μg from T4 infected cells (*C*). The reactants were incubated as for Table 1 at 37° C, but 25 μl. aliquots were withdrawn at the stated times and diluted into 3 ml. of buffer A, whereupon they were filtered and counted in the normal manner. After 7 min 420 μg of *E. coli* factors was added to *A* and *C*; and 400 μg of T4 factors to *B*. The subsequent binding of fMet-tRNA$_f$ to ribosomes (in pmoles) was followed as a function of time. Results from control experiments containing no factors have been subtracted from the total binding of fMet-tRNA$_f$.

the ribosome. While for both T4 and host mRNAs these are average results from a spectrum of messengers isolated between 12S and 18S on a sucrose density gradient, the same results were also obtained when fractions across this gradient were assayed.

Similar conclusions have been drawn from experiments in which the initiation complex itself was isolated on sucrose density gradients[21]. Both ^{32}P-labelled and non-radioactive messengers were isolated and combined, so that the binding capabilities of fMet-tRNA$_f$ and messenger RNAs could be investigated independently (Fig. 1). There is a small residual ribosomal binding of both fMet-tRNA$_f$ and R17 or host messengers with T4 factors and *E. coli* washed ribosomes, which cannot be abolished even with washed ribosomes and factors from T4 infected cells. This residual ribosomal binding with R17 RNA is the subject of the following communication[14].

Because we detected some nucleolytic breakdown of the R17 RNA with T4 factors and ribosomes, as a control we repeated the normal ribosomal binding reaction with the complete system containing T4 derived ribosomal components, then reisolated the RNA, and used this in a subsequent binding reaction with host cell ribosomal components. There was virtually no change in the amount of fMet-tRNA$_f$ or mRNA bound to the ribosome compared with a control system starting with *E. coli* ribosomal components in the first reaction mixture, both for the host mRNAs (Fig. 1*e*) and R17 RNA. Consequently the extra nucleolytic activity is not the primary cause for the failure of T4 factors to support a stable initiation complex with host messengers.

The relationship of the messenger-discriminating agent associated with ribosomes from T4 infected cells to existing ribosomal components is not clear. As shown in Table 2, all messengers required a component additional to the initiation factor proteins F1 and F2a, whereas the initator codon AUG did not. This protein, which assists natural mRNA binding, has been termed F3 (ref. 22) or B (ref. 23). Exchange, modification or destruction of the host's F3 molecules on phage infection might result in preferential selection of phage mRNAs over those of the host.

Table 2 also shows that the inability of F1 and F2a to support a strong initiation complex with natural messengers is maintained even after re-extraction of the RNA from a reaction mixture containing ribosomal components from T4 infected cells and host ribosomes. Thus the nuclease activity of these components was not exposing additional initiator codons (that is, AUG or GUG) which could serve as fresh binding sites.

Factor proteins from T4 infected cells compete with those from normal cells and destroy the initiation com-

Table 2. RIBOSOMAL BINDING OF mRNAs WITH INITIATION FACTORS F1 AND F2a

Host factors	T4 factors	F1 + F2a factors	ApUpG	R17 RNA		T4 mRNAs		Host mRNAs		Host mRNAs*	
			[^{3}H]	[^{3}H]	[^{32}P]	[^{3}H]	[^{32}P]	[^{3}H]	[^{32}P]	[^{3}H]	[^{32}P]
−	−	−	1,500	300	200	1,300	800	700	1,500	−	−
+	−	−	14,200	11,000	8,100	15,800	8,800	12,100	12,700	−	−
−	+	−	14,600	500	300	13,300	7,900	1,600	1,300	−	−
−	−	+	14,100	1,100	300	1,100	600	500	1,600	700	1,000

* Host mRNAs extracted from an incubation mix containing T4 factors.
[^{3}H] refers to ^{3}H-fMet-tRNA$_f$, and [^{32}P] to ^{32}P-mRNA.

Initiation factors F1 and F2 of Stanley *et al.*[32] were further purified by fractional precipitation with $(NH_4)_2SO_4$ and passage over a phosphocellulose column for F1[33], or chromatography on successive columns of hydroxyapatite and 'DEAE-Sephadex' for F2 (unpublished results of P. S. R.). The original F2 fraction was resolved into two components, termed F2a and F2b in order of elution from the initial DEAE-cellulose column. Reaction mixtures (50 μl.) and procedure are as for Fig. 2 except that either no factors, *E. coli* factors, or T4 factors were added in the same amounts as used previously. In addition 4 μg of F1 and 80 μg of F2a were added as indicated in place of the unfractionated factors. Messengers used were in the same concentrations as for Fig. 2 and also 0·14 $A_{260\ nm}$ units of ApUpG were included. The total radioactivity in the 70S region of the gradient was tabulated for both ^{3}H-fMet-tRNA$_f$ and ^{32}P-mRNA. In the final column, experiment (*e*) from Fig. 1 was repeated by incubating host mRNAs with T4 factors and *E. coli* ribosomes, but the RNA obtained after phenol extraction was reincubated in the complete reaction mixture with F1 and F2a at the same concentrations as above, in place of the unfractionated factors.

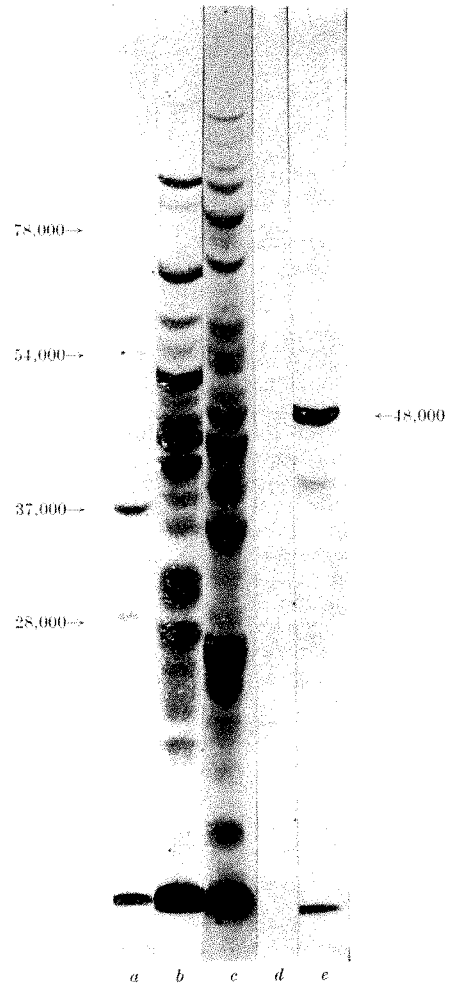

Fig. 3. Autoradiograms of polyacrylamide gels. Disc electrophoresis[24] in 10 per cent polyacrylamide gels containing 0·1 per cent sodium dodecyl sulphate[25], as well as the preparation of autoradiograms[26], was carried out as described by U. Laemmli and J. Maizel (manuscript in preparation). *a*, Factor fraction from T4 infected cells labelled with ^{35}S from 2 to 7 min after infection; *b*, factor fraction from uninfected cells labelled with ^{35}S for 5 min; *c*, 100,000*g* ribosome free supernatant from T4 infected cells pulsed from 2 to 7 min; *d* and *e*, proteins from 2 M NH_4Cl washed ribosomes from T4 infected cells, pulse time 5 to 10 min after infection *d* and 10 to 15 min after infection *e*. In addition to the major protein bands there is some general background. The molecular weights were determined with reference to the mobilities of marker proteins, and are accurate to within 10 per cent. *E. coli* MRE600 cells were grown at 37° C in 100 ml. of low sulphate medium containing 0·02 M Na_2HPO_4; 0·011 M KH_2PO_4; 0·047 M NaCl; 0·009 M NH_4Cl; 0·00095 M $MgCl_2$; 0·00005 M $MgSO_4$; and 0·4 per cent glucose. At a cell density of 3×10^8 per ml., cells were infected with T4 phage as described in Table 1 and pulsed for 5 min with $^{35}SO_4$ at (*a*) 2, (*c*) 5, (*d*) 10 min after infection. A preparation of ^{35}S-labelled uninfected cells (*b*), pulsed for 5 min in the same conditions, was also made. Isolation of ribosomes and factors was as described in Table 1, except that ribosomes were first sedimented through a buffer containing 0·001 M puromycin to remove nascent polypeptide chains, before sedimenting through the 2 M NH_4Cl buffer.

plex formed with the host mRNAs almost completely. Consequently, equal amounts of host factors are unable to restore the ribosomal binding ability in the reaction mixture containing *E. coli* washed ribosomes and factors from T4 cells, as measured by the ribosomal binding of fMet-$tRNA_f$ on nitrocellulose filters (Fig. 2). Partial restoration of the initiation complex on sucrose density gradients was observed, however, on addition of a five-fold excess of host factors, possibly reflecting a higher affinity of T4 factors for the ribosome.

New Proteins in T4 Factor Fraction

Finally, we pulse-labelled the host cells with radioactive sulphate following T4 infection, and fractionated the proteins obtained from them on polyacrylamide gels. Several new proteins were found in the ribosomal factor fraction and a major one associated with salt washed ribosomes (Fig. 3). The former could be detected within 2 min of phage infection and were still present 15 min later, while the latter was not apparent until 10 min after infection. None of the new proteins exhibits the same mobility as any of the host factor proteins or of the 100,000*g* supernatant proteins synthesized after T4 infection. Nevertheless, the possibility that these new proteins arose from modifications of existing ones cannot be discounted. (Smith and Haselkorn quoted in ref. 12 have also found new proteins associated with ribosomes after T4 infection.)

The relevance of these proteins to the functional alteration of ribosomes is not yet clear, but it may be significant that new proteins in the factor fraction can be detected within the time period of termination of host protein synthesis. In addition, the finding that T4 factors hinder the *in vitro* initiation complex formation of f2 and R17 as well as of host mRNAs may explain in part observations *in vivo* on the restriction of viral (R17 and f2) and host protein synthesis after T4 phage infection.

In conclusion, the results demonstrate a possible mechanism for translational control of gene expression in the infected cell, in that selection for a class of messengers is imposed after infection. This selection operates at the level of polypeptide chain initiation, and the agent(s) primarily responsible occur(s) in the ribosomal factor fraction. It would not be surprising if more elaborate mechanisms of this general nature occur in higher organisms.

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Control of Translation by T4 Phage: Altered Ribosome Binding at R17 Initiation Sites

by

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After infection of *E. coli* by T4 phage, ribosomes lose their ability to bind efficiently to the coat and synthetase initiation sites of R17 RNA. This change lies in the initiator factor fraction.

THE preceding article[1] shows that ribosomes isolated from *Escherichia coli* infected with phage T4 interact only weakly with phage R17 RNA to form polypeptide chain initiation complexes *in vitro*. To examine the messenger sites recognized by these ribosomes, we have made use of the fact that ribosomes from uninfected *E. coli* attach to the initiator regions of all three genes in the R17 RNA. Ribosomes from T4 infected cells, by contrast, discriminate against the coat protein and synthetase initiation sites and bind appreciably only to the beginning of the A protein cistron. This property, like the agent affecting the levels of initiation complex formation described in the preceding article, resides in the initiation factor preparation.

If radioactive R17 RNA is incubated with ribosomes from *E. coli* in the presence of GTP and formylmethionyl tRNA, regions at the beginning of its three cistrons are bound and protected from pancreatic ribonuclease digestion[2]. A sequence of approximately thirty-five nucleotides at each of these sites has been elucidated. When isolated from the 70S ribosomal fraction of a sucrose gradient and subjected to "fingerprint" analysis, mixtures of the three R17 initiator fragments yield several large T_1 oligonucleotides (Fig. 1*a*). Because each of these (with the exception of UUUG) arises specifically from one or another of the three R17 binding sites, a T_1 ribonuclease fingerprint provides a direct assay for differential ribosome recognition of the initiator regions in a messenger RNA. The relative yields of the three sites can be estimated not only by inspection, but determined

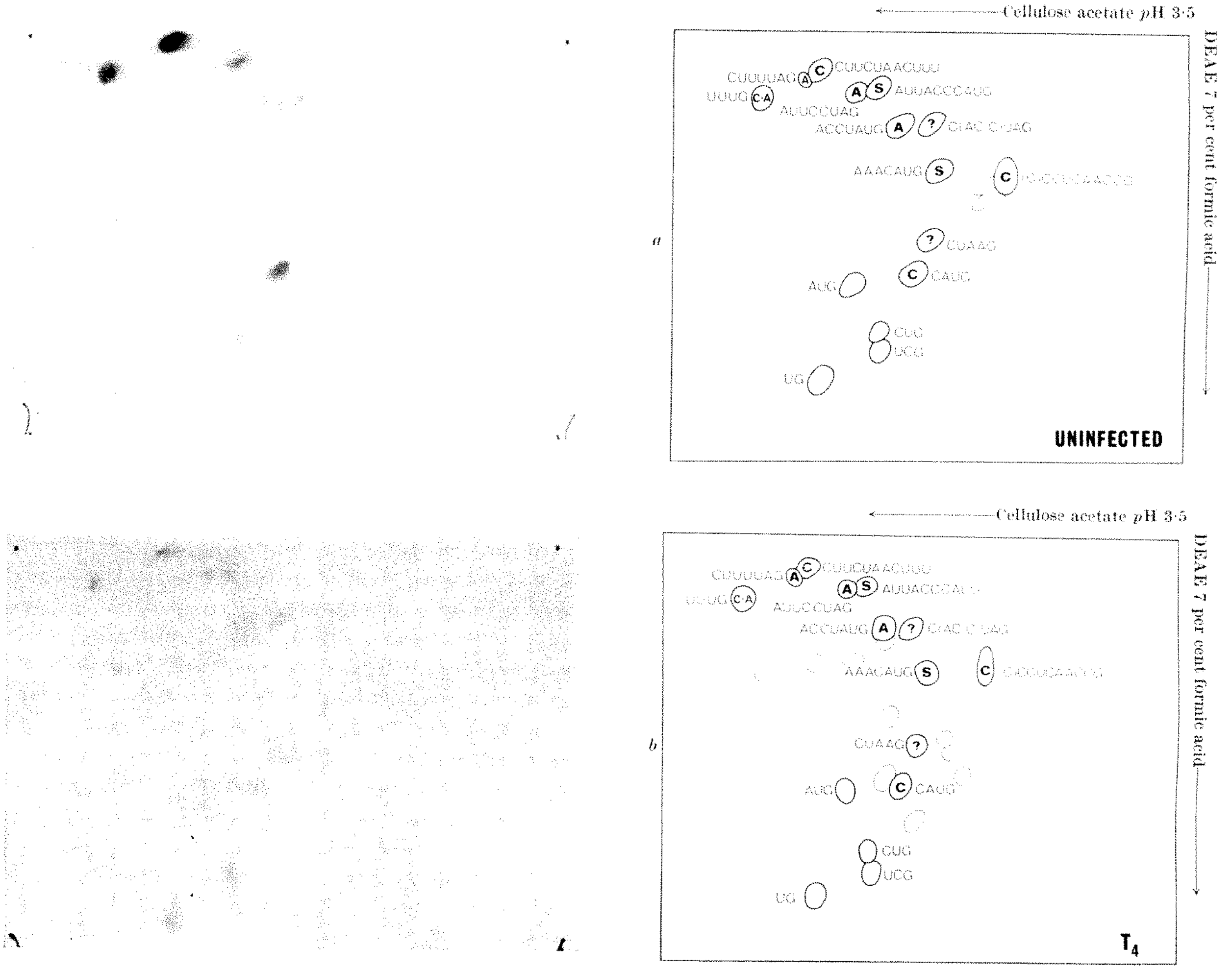

Fig. 1. Ribonuclease T1 fingerprints of R17 initiator regions bound by low-salt-washed ribosomes from uninfected (*a*) and from T4-infected (*b*) cells. The fingerprints shown are those obtained in experiment 1 of Table 2, where the details of the binding reactions and of the preparation of the ^{32}P-labelled RNA fragments for fingerprinting are given. Fingerprint *a* was exposed for 36 h whereas fingerprint *b* was exposed for 5 days. The illustrations exemplify the type of fingerprints from which oligonucleotide spots were cut and counted to provide the data in Tables 1–3. The diagrams of the fingerprints show the sequences of those oligonucleotides routinely analysed and their origins: A, a protein initiation site; C, coat protein site; S, synthetase site. The two oligonucleotides marked "?" have been frequently detected (in varying amounts) in T_1 ribonuclease fingerprints of R17 binding sites, but they have never been found linked to any of the three established sequences.

quantitatively by counting the radioactivity in the appropriate oligonucleotide spots (which can subsequently be positively identified by analysis of their pancreatic ribonuclease digestion products).

The fingerprint in Fig. 1*a* and the data in Table 1 (experiment 1, column *a*) give the results of one binding assay in which the A, coat and synthetase initiation sites were protected in molar ratios of 1:7·3:1·6 by ribosomes washed at low salt concentration (which retain their initiation factors) from uninfected *E. coli*. The relative amounts of the three sites vary from experiment to experiment depending on the integrity of the ^{32}P-labelled RNA added to the binding reaction; more degraded R17 RNA results in lower yields of the coat site relative to the A and synthetase sites[2], as in experiment 2, Table 1, column *a*. In parallel binding reactions performed with the same R17 RNA and ribosome preparations, however, a variability of only about 30 per cent in the ratios of the three sites is observed (although the absolute number of counts obtained in duplicate fingerprints may be quite different because of differential losses during the preparation of samples for fingerprinting).

Differential Recognition of R17 RNA Binding Sites

A T$_1$ ribonuclease fingerprint of R17 initiator regions bound by low-salt-washed ribosomes from cells harvested 13 min after infection with T4 phage is illustrated in Fig. 1*b*. Here the ratios of the oligonucleotides corresponding to the three binding sites (Table 1, experiment 1, column *b*) are strikingly different from those obtained in the parallel reaction with ribosomes from uninfected cells (column *a*). The A protein is initiated as efficiently as the coat protein; the recoveries of the coat and synthetase binding sites relative to the A have diminished by factors of seven and four, respectively. In experiment 2, performed with the more degraded RNA preparation, a similar effect is observed, confirming that the results are independent of the integrity of the R17 RNA used for the binding assay.

A lower absolute level of binding (even at the A site) is consistently obtained with ribosomes from T4-infected cells. In addition, not all the labelled RNA recovered from these ribosomes can be attributed to the three known R17 binding sites. The several new spots are of unknown origin and, with a few exceptions, have not been previously detected. They are unlikely to be degradation products of the missing coat and synthetase oligonucleotides as demonstrated by a control experiment discussed later (Table 2, column *e*).

To find which component determines the proportions of the three R17 initiation sites bound, ribosomes from uninfected and T4-infected *E. coli* were washed with 2 M NH_4Cl buffer to remove their initiation factors[3]. The washed ribosomes and initiation factor preparations were then tested in various combinations (Table 2). Both experiments show that the recognition pattern of the three initiation sites in the R17 RNA is determined by some component(s) of the initiation factor fraction; the ratios obtained in the mixed infected–uninfected binding reactions (columns *b* and *c*) reflect only the origin of the factor preparation used. A lower absolute level of binding seems to occur when ribosomes from T4-infected cells are used; in light of the uncertain overall recovery of RNA fragments and the data presented in the preceding article[1], this observation is of questionable significance.

Table 1. RELATIVE AMOUNTS OF R17 INITIATION SITES BOUND BY LOW-SALT-WASHED RIBOSOMES FROM UNINFECTED AND T4-INFECTED CELLS

	(*a*) Ribosomes from uninfected cells	(*b*) Ribosomes from T4-infected cells	(*c*) Ribosomes from T4-infected cells, then ribosomes from uninfected cells
Experiment 1			
A protein			
ACCU*AUG*	1(= 111 c.p.m./P)	1(= 18 c.p.m./P)	1(= 34 c.p.m./P)
Coat protein			
C*AUG*	7·3	1·0	3·7
CUUCUAACUUU	5·9	0·60	3·2
Synthetase			
AUUACCC*AUG*	1·6	0·43	1·2
Experiment 2			
A protein			
ACCU*AUG*	1(= 103 c.p.m./P)	1(= 68 c.p.m./P)	
Coat protein			
C*AUG*	2·6	< 0·36*	
CUUCUAACUUU	2·3	< 0·11*	
Synthetase			
AUUACCC*AUG*	0·86	0·22	

In experiment 1, parallel binding reactions were performed at 37° C in 70 μl. of solution containing 0·05 M ammonium cacodylate (*p*H 7·0), 0·1 M NH_4Cl, 0·01 M β-mercaptoethanol, 0·0003 M GTP, 0·006 M magnesium acetate, 5·5 $A_{260\ \mathrm{nm}}$ units of charged-formylated mixed tRNA, 1·2 $A_{260\ \mathrm{nm}}$ units of freshly prepared ^{32}P-labelled R17 RNA (specific activity about $1·5 \times 10^6$ c.p.m./μg), and 10 $A_{260\ \mathrm{nm}}$ units of (*a*) ribosomes from uninfected MRE600, (*b*) ribosomes from MRE600 infected with phage T4 (ref. 1), or (*c*) ribosomes from T4-infected cells for 7 min, then an additional 10 $A_{260\ \mathrm{nm}}$ units of ribosomes from uninfected cells for 8 min. In experiment 2, reactions were performed in 100 μl. containing the same concentrations of components as above, except: 6·6 $A_{260\ \mathrm{nm}}$ units of charged-formylated mixed tRNA, 3·0 $A_{260\ \mathrm{nm}}$ units of 2 week old ^{32}P-labelled R17 RNA (specific activity about 4×10^5 c.p.m./μg) and 15 $A_{260\ \mathrm{nm}}$ units of ribosomes from (*a*) uninfected MRE600, or (*b*) MRE600 infected with T4 phage. Transfer RNA and ^{32}P-labelled R17 RNA were prepared as described previously[3]. Ribosomes from MRE600 and from MRE600, collected 13 min after infection with T4 phage, were prepared by washing three times with buffer A[15], as described in the accompanying paper[1]. After 10 min of incubation—or as noted above for (*c*)—reaction mixtures were cooled to room temperature, digested for 15 min after the addition of 5 μg/ml. pancreatic ribonuclease, and sedimented in sucrose gradients as described in ref. 2. Fractions from the 70S ribosome peak were pooled and extracted with phenol in the presence of 0·2 M Tris-HCl (*p*H 7·4) and 0·1 per cent sodium dodecyl sulphate. Following several ethanol precipitations, the RNA was digested with T1 ribonuclease and fingerprinted as described[2]. After autoradiography, the oligonucleotide spots were cut from the papers and counted for 20 min in toluene scintillator. Each was then rinsed with toluene, eluted, digested with pancreatic ribonuclease and fractionated on DEAE-paper at *p*H 3·5 according to standard procedures[16]. For simplicity we present in the tables the molar ratios only of the initiator AUG-containing oligonucleotides; c.p.m. observed per phosphate for the A-site oligonucleotide ACCUAUG have been set equal to 1. Nevertheless, every spot marked A, C, or S in Fig. 2 was routinely counted and analysed, providing additional data for evaluation. In the case of the coat protein initiator region, CUUCUAACUUU is required in addition to the initiator AUG-containing oligonucleotide to define the site, for the sequence CAUG is not long enough to have a high probability of being unique in the R17 RNA molecule. CUUCUAACUUU, however, is always obtained in lower yields than CAUG because it arises from the 3′ end of the coat initiator fragment[2]. Where indicated by *, the oligonucleotide spots were found to contain pancreatic ribonuclease digestion products other than those expected, comprising greater than 50 per cent of the radioactivity; the values given have therefore been reduced to half the observed. All other oligonucleotides proved to be more than 90 per cent pure.

Does Specific Messenger Degradation Occur?

Altered recognition of the three R17 initiation sites with factor preparations from T4-infected cells could result from selective degradation of the coat and synthetase binding sites. Both low-salt-washed ribosomes and factor preparations from infected cells contain considerably more nucleolytic activity towards R17 RNA than comparable fractions from uninfected cells[1]. We therefore carried out several experiments which eliminate this explanation of the results.

First, we tested the effect of incubating R17 RNA in a complete binding system with low-salt-washed ribosomes from T4-infected cells before adding ribosomes from uninfected *E. coli* (Table 1, column *c*). The coat and synthetase oligonucleotides are recovered in quite good yield (compare with column *a*), showing that the inability of ribosomes from T4-infected cells to attach to these sites (column *b*) is not the result of selective messenger destruction.

Second, we have shown that if ^{32}P-labelled R17 RNA is preincubated in a binding mixture including factor preparations from T4-infected cells (with or without ribosomes) and then re-extracted with phenol before its addition to a reaction containing ribosomes and factors from uninfected cells (Table 3, columns *b* and *c*), the amounts of the coat and synthetase sites bound relative to the A initiator region are similar to the control (column *a*). Thus, in the fractionated system too, selective degradation of coat and synthetase sites does not occur. This type of experiment was necessary because we were unable to recover the coat and synthetase sites simply by adding equal amounts of factors from uninfected cells after incubation of R17 RNA in the presence of T4 factor prepa-

Table 2. RELATIVE AMOUNTS OF R17 INITIATION SITES BOUND USING SEPARATED RIBOSOMES AND INITIATION FACTORS

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
Factors:	Uninfected	Uninfected	T4-infected	T4-infected	Uninfected, then T4-infected	T4-infected, then uninfected
Ribosomes:	+ uninfected	+ T4-infected	+ uninfected	+ T4-infected	+ uninfected	+ uninfected
Experiment 1						
A protein						
ACCU*AUG*	1(= 80 c.p.m./P)	1(= 20 c.p.m./P)	1(= 6·3 c.p.m./P)	1(= 6·1 c.p.m./P)		
Coat protein						
C*AUG*	10	8·2	< 0·7 ‡	< 0·9 ‡		
CUUCUAACUUU	3·8	4·1	< 0·4 ‡	< 0·34*		
Synthetase						
AUUACCC*AUG*	1·5	1·8	0·29	0·31		
Experiment 2						
A protein						
ACCU*AUG*	1(= 130 c.p.m./P)	1(= 24 c.p.m./P)	1(= 17 c.p.m./P)	1(= 20 c.p.m./P)	1(= 61 c.p.m./P)	1(= 39 c.p.m./P)
Coat protein						
C*AUG*	4·7	2·3	< 0·4 †	< 0·4 ‡	4·4	< 0·3 ‡
CUUCUAACUUU	4·0	2·4	< 0·46*	< 0·14*	3·6	0·14
Synthetase						
AUUACCC*AUG*	1·4	1·3	0·22	0·22	0·85	0·38

* Oligonucleotides found upon analysis to contain somewhat more than 10 per cent contaminants.
† Oligonucleotides containing more than two-thirds contaminants; the value given has therefore been reduced to one-third the observed.
‡ Oligonucleotides containing more than one-half contaminants; the values given have therefore been reduced to half the observed.

In both experiments, parallel binding reactions were performed at 37° C in 70 μl. of solution containing buffers and salts as in Table 1, 6·5 $A_{260\,\text{nm}}$ units of charged-formylated mixed tRNA, 1·5 $A_{260\,\text{nm}}$ units of ^{32}P-labelled R17 RNA (from the same preparation, specific activity about $1\cdot5 \times 10^{6}$ c.p.m./μg in experiment 1 and $1\cdot2 \times 10^{6}$ c.p.m./μg in experiment 2), 10 $A_{260\,\text{nm}}$ units of high salt-washed ribosomes and 200 μg of initiation factors from uninfected or T4-infected MRE600 as indicated. Ribosomes were washed twice overnight in buffer B[15]; initiation factors were prepared from the first wash and titrated against the ribosomes as described previously[1]. Incubations were for 10 min in reactions (*a*) to (*d*); in (*e*) factors from uninfected cells were present for 8 min before adding additional factors from T4-infected cells and incubating a further 10 min; in (*f*) factors from T4-infected cells were present for 8 min before adding additional factors from uninfected cells and incubating a further 10 min. Pancreatic ribonuclease digestion, isolation and fingerprinting of the radioactive fragments, and quantitation and analysis of the oligonucleotide spots were performed as described in Table 1. All oligonucleotides were more than 90 per cent pure except as indicated in the footnotes.

Table 3. RELATIVE AMOUNTS OF INITIATION SITES BOUND BY RIBOSOMES FROM UNINFECTED CELLS USING R17 RNA PREVIOUSLY INCUBATED IN THE PRESENCE OF SEPARATED RIBOSOMES AND INITIATION FACTORS

RNA preincubated with:	(*a*) Ribosomes and factors from uninfected cells	(*b*) Ribosomes and factors from T4-infected cells	(*c*) Factors from T4-infected cells
A protein			
ACCU*AUG*	1(= 10·5 c.p.m./P)	1(= 8·9 c.p.m./P)	1(= 22·4 c.p.m./P)
Coat protein			
C*AUG*	2·3	1·7	1·4
CUUCUAACUUU	1·1	1·0	0·8
Synthetase			
AUUACCC*AUG*	1·2	1·8	1·4

Preincubation was performed in three complete binding reactions (70 μl.) as described in Table 2. The ^{32}P-labelled RNA used was 2 weeks old and had a specific activity of about 8×10^{5} c.p.m./μg; ribosomes and factors were as indicated in Table 1. Note that no ribosomes were present in (*c*). After 10 min at 37° C, the reaction mixtures were cooled and extracted with phenol following the addition of 1 ml. of 0·2 M Tris-HCl (*p*H 7·4), 0·1 per cent sodium dodecyl sulphate. The RNA from each of the resulting aqueous layers was ethanol precipitated twice and then redissolved in 5 μl. of 10^{-3} M EDTA before reincubation for 10 min at 37° C in a 70 μl. binding reaction containing ribosomes and factors from uninfected cells (concentrations of all components as in Table 2). Ribonuclease digestion of the complexes and subsequent isolation and analysis of the bound initiator fragments were as described in Table 1. Although the relative amount of coat site bound is low (because of the degraded RNA used) in three assays, it is not significantly different from experiment 2, Table 1, where it dropped by a factor of seven with ribosomes from T4-infected cells. On analysis, all oligonucleotides were more than 90 per cent pure.

rations (Table 2, column *f*). Taken together, it seems that recognition of the coat and synthetase sites may be blocked by some component of the initiation factor fraction from T4-infected cells, which is present in higher concentrations when the fractionated system rather than low-salt-washed ribosomes are used. Such a possibility has not been investigated further.

One additional control experiment (Table 2, column *e*) provides evidence that T4-specific degradation of T_1 oligonucleotides derived from the coat and synthetase initiator regions does not occur during isolation and preparation of the radioactive messenger fragments for fingerprinting. The relative ratios of the three sites are not greatly altered (compared with column *a*) when factors from T4-infected cells are added after preincubation of R17 RNA with ribosomes and factors from uninfected *E. coli*.

Initiation Factors and Control

A high-salt-wash fraction from ribosomes of *E. coli* contains many proteins, several of which are required for the initiation of protein synthesis[3]. Two factors[4], F1 and F2, are required for the initiation of polypeptide chains in the presence of synthetic polymers, and a third (variously characterized) factor is also necessary for initiation by natural messenger RNAs[4,5].

The altered initiation site recognition observed with ribosomes from T4-infected cells may result from a phage-coded modification or replacement of one or more of the known factors or of some unknown component(s) in the initiator factor fraction. The change induced by T4 seems to have two effects. First, the overall level of R17 initiation sites bound to ribosomes is decreased, as demonstrated in the previous article 1. This presumably is reflected by the inefficiency of RNA phage genomes to act as messengers in extracts of T4-infected cells (personal communication from W. Salser and R. F. Gesteland, and refs. 6 and 7). Second, and more important, the proportion of the three R17 sites recognized by ribosomes from T4-infected cells is changed in favour of the A site. Although this could conceivably result from an altered common initiation factor, a most attractive possibility is that multiple site-specific initiation factors exist in *E. coli* which are modified or replaced to varying degrees after infection by T4 phage. Were there several such factors, it would not be difficult to understand why protein synthesis is initiated at the three R17 cistrons with very different efficiencies *in vitro* (molar ratios of 20:6:1 for coat : synthetase : A protein, for instance, have been observed[8]). A multiplicity of factors recognizing different initiator sequences might also explain why the three ribosomal binding sites in the R17 RNA do not possess a common sequence which could act as a special signal for polypeptide chain initiation[2].

In conclusion, it seems not unlikely that in addition to changes at the level of transcription[9-11] modification or substitution of initiation factors may be a device used by virulent phages to turn the host's protein synthetic machinery to their own advantage.

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Interpretation of Cosmic X-ray and Gamma Ray Spectra

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Changes in the slope of a measured distribution may be explained partially by the effect of the measurement process itself.

MANY papers published recently consider the cosmological implication of the diffuse X-ray and gamma ray background[1-4]. Certain breaks in the slope of the measured spectra have been noted, and some theoretical models have been proposed to explain these changes. These changes in slope may be explained partially by the effect of the measurement process itself.

The measured pulse heights reported are not identical with the true photon spectra (which are of theoretical significance), because the response of a real crystal detector to a delta-function photon spectrum is not a delta-function pulse height spectrum but is a characteristic continuous response function describing the energy imparted to the crystal. Before we compare theory with experiment, with respect to the measured pulse height spectra, the detector effects should be eliminated from these spectra.

Information concerning the differential energy photon spectrum is obtained by measuring the interaction of these photons with a detector system. This measured interaction spectrum, or pulse height spectrum*, does not necessarily reflect a one-to-one correspondence with the photon spectrum. Consider equation (1)

$$Y(V)=\int_{0}^{E_{\max}} T(E)S(E,V)\mathrm{d}E \qquad (1)$$

where $T(E)$ is the differential energy photon spectrum as a function of E, $S(E,V)$ is the detector interaction function which reflects the mechanisms of converting photons of energy, E, into a pulse height signal, V, at the output of the detector, and $Y(V)$ is the measured pulse height or interaction spectrum as a function of pulse height, V.

The analysis of gamma ray pulse height spectra in this particular spectral region must be accomplished by numerical methods because we cannot yet perform this inverse transform analytically. The integral in equation (1) can be written as a sum of discrete components. $T(E)$ is not a discrete distribution in energy, but a continuous energy distribution. The method for obtaining $T(E)$ is based on a theorem in sampling theory[5-7] and on the fact that there is a finite energy resolution of the detection system. If a distribution has no oscillatory component with a frequency greater than $f_{\max}$, then, the Shannon Sampling Theorem asserts, samples at discrete points not farther apart than $1/2\,f_{\max}$ describe the original function exactly. The original function may be reconstructed from the samples[6]. Thus equation (1) can be written as

$$Y_i=\sum_{j} T_j S_{ij} \qquad (2)$$

where Y_i is the measurement of $Y(V)$ in channel or pulse height i, T_j is the total number of gamma rays in energy group ΔE_j about E_j, and S_{ij} is the value of the jth standard spectrum in channel i. The S_{ij} components are the response of an NaI(Tl) detector to an incident monoenergetic gamma ray.

In applying Shannon's Sampling Theorem to the analysis of spectra obtained with NaI(Tl) crystals, sampling components S_{ij} are chosen so that their separation energy is no greater than the half width at half maximum of their photopeaks. Further, the integral in equation (1) is a sum of discrete energy components which describe an energy interval, ΔE, about a given E; the energy intervals between the ΔE samples are less than the half width at half maximum of the photopeak for the function, $S(E,V)$ (ref. 7). Thus the approximation formula, equation (2), is obtained. Equation (2) is now in a form where a valid numerical transformation can be obtained using techniques derived from the analysis of variance. The details of this derivation can be found in ref. 8 and unpublished work of J. I. T. and Schmadebeck. Using the least square principle, the method requires that

$$M=\sum_{j}\omega_i\Big(Y_i-\sum_{j}T_jS_{ij}\Big)^2 \qquad (3)$$

be a minimum, where ω_i is the statistical weight corresponding to the measurement of Y_i on channel i, and $\omega_i=1/\sigma_i^2$ (where σ_i^2 is the variance of the count in channel i). The other terms have been defined. The partial derivations of M are taken with respect to T in order to determine the minimum. The solution in matrix form is given by equation (4)

$$T=(\tilde{S}\omega S)^{-1}\tilde{S}\omega Y \qquad (4)$$

where Y is a vector describing the pulse height spectrum, S is an $m\times n$ matrix describing the discrete set of detector response function using the selection rule derived here, $\tilde{S}$ is the transposition of S, ω is a square diagonal matrix of the weighting functions, and T is a best estimate of the differential energy photon spectrum.

We now present some illustrations showing the nature of the problem and solutions that can be obtained. Assume that we are trying to measure a photon spectrum, $T(E)$, which can be described by a differential energy distribution, $T(E)\ \mathrm{d}E=E^{-1.5}\ \mathrm{d}E$. Fig. 1*a* shows such a distribution, and $T(E)\ \mathrm{d}E$ is indicated.

The spectrum is measured using a 3 × 3 inch NaI(Tl) crystal. The pulse height spectrum, $Y(V)\mathrm{d}V$, that would be obtained using equation (2) is indicated in Fig. 1*a*. If we attempt to determine the slope of the line of the pulse height spectrum, we find the slope to be $Y(V)\mathrm{d}V=V^{-2.3}\mathrm{d}V$, significantly different from $\boldsymbol{T(E)}$. Further, if we measure the pulse height spectrum only in the region up to about 60 keV, $Y(V)\mathrm{d}V=V^{-1.5}\mathrm{d}V$. If we measure the spectrum from 100 to about 300 keV, $Y(\mathrm{d}V)V = V^{-1\cdot7}\mathrm{d}V$, and $Y(V)\mathrm{d}V = \mathrm{d}V^{-2\cdot3}\mathrm{d}V$ if we determine the slope from 300 keV to 2 MeV. The shape of $Y(V)$ will depend on the size of the detector used, the resolution of the detector, the angular distribution of the incident and other factors. More important, if we assume some type of distribution like the photon inelastic scattering process proposed in ref. 1 (Fig. 1*b*) is superimposed on this power law distribution there is a deviation of $T(E)\mathrm{d}E$ from the power law distribution at higher energies. The detection system damps out this deviation in the photon spectrum, $T(E)$, and we lose the information concerning this power law process if we analyse the pulse height spectrum alone. This damping depends, again, on factors of crystal size, resolution and the angular distribution of the incident flux. Thus almost any kind of solution may be obtained if the pulse height spectrum is mistakenly used for correlating theory with experiment. Fig. 2 shows both the measured pulse height spectrum (*a*) and the analytically derived photon spectrum (*b*) obtained using the analytical method just described. The measured pulse height spectrum (Fig. 2*a*) was obtained during a balloon flight at approximately 100,000 feet, using a 3 × 3 inch NaI(Tl) detector (private communication from Peterson). The 0·51 MeV annihilation ray peak is easily recognized in the pulse height spectrum. Without assuming any *a priori* knowledge of the incident photon spectrum, the numerical transformation was performed and the photon spectrum obtained. After the numerical analysis, two other discrete lines became more distinct above background. These lines were later identified as the potassium (^{40}K) line caused by the presence of this material in the atmosphere, and the magnesium (^{24}Mg) line attributed to excitation induced by the cosmic ray flux on the structural material of the gondola. Once identified, these discrete lines were subtracted from the total photon spectrum to obtain the pure continuous spectrum.

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* The term pulse height is used to describe the spectra, because the detector output is usually an analogue voltage or current signal. This analogue signal is digitized using a multichannel pulse height analyser.

The photon spectrum (Fig. 2*b*) was used to reconstruct the pulse height spectrum (equation 2). The results are shown in Fig. 2*a*. It is almost impossible to differentiate between the two curves.

In Fig. 2*b* the last two points are higher than the continuum. These two points contain information about all the energies greater than those observed in the measured energy region. These "end effects" occur when only parts of the total spectrum are analysed.

In summary, we have developed a method to determine both the pulse height spectrum from a known photon spectrum and the photon spectrum from a measured pulse height spectrum, and information derived from these two spectra differs significantly. Using a 3 × 3 inch NaI(Tl) detector, if we attempt to measure a photon differential energy spectrum characterized by a $T(E)\mathrm{d}E = E^{-1\cdot5}\mathrm{d}E$ distribution, we obtain a pulse height spectrum with a number of breaks in slope: a $-1\cdot5$ slope in the energy region up to 60 keV, a $-1\cdot7$ slope in the energy region up to 300 keV, and a slope of $-2\cdot3$ from 0·3 MeV to 2 MeV. The technique for deriving the photon spectra from the measured pulse height spectra eliminates the effects of instrumental response, and further allows for meaningful comparison with data from different detectors.

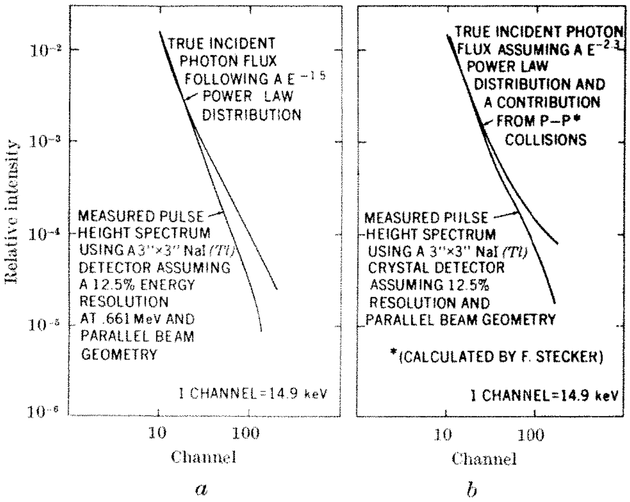

Fig. 1. Effect of detector interaction with incident photon flux in the measurement of gamma rays.

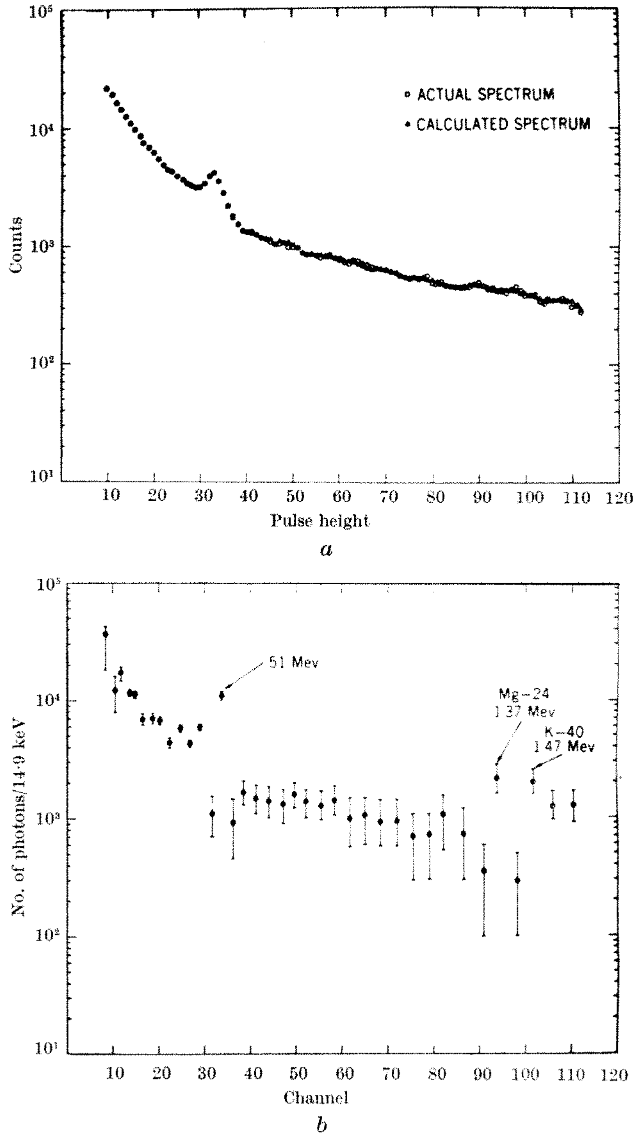

Fig. 2. *a*, Pulse height spectrum. 3 × 3 inch NaI(Tl) crystal atmospheric spectrum. *b*, Photon spectrum. 1 channel = 14·9 keV.

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LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Telemetry from the First Chinese Artificial Earth Satellite

THE first Chinese artificial Earth satellite was launched at approximately 1330 UT on April 24, 1970. The announcement of the launch, giving a transmission frequency of 20·009 MHz, was made in Peking at 1232 UT on April 25. First signals were received at Kettering Grammar School at 1402 UT, towards the end of the twelfth orbit of the Earth.

As announced later in Peking, the signals consist of a 1 min cycle of alternate music and telemetry[1]. The first 40 s are devoted to a repetition of the music of "Tungfanghung"—"The East is Red" (Fig. 1)—followed by an interval of 5 s. Then follows 10 s of telemetry and, after a further interval of 5 s, the complete sequence is repeated.

The telemetry consists of audio tones which seem to be quantized in steps of 13·6 Hz. The first tone has the same frequency as the highest musical tone. The duration of the first tone is twice that of succeeding tones and its frequency is 100 times the minimum difference between tones, so it is considered to serve as a calibration point nominally equal to 100 per cent. Then follow twenty tones of equal duration, terminated by several rapid tones.

Values of the initial tone and twenty channels, expressed as a percentage of the maximum observed frequency, are given for revolutions 24 and 99 in Fig. 2. To date, no frequency below 68 per cent has been observed. Seven of the channels had this minimum value on revolution 99 and this prompts speculation that transducer outputs are quantized in 32 levels before transmission. The fact that other values cluster around 84 per cent (or level 16) supports such speculation. Read-out as a five-bit binary code for storage and data processing is therefore a possibility.

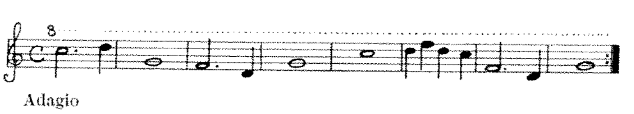

Fig. 1. "Tungfanghung."

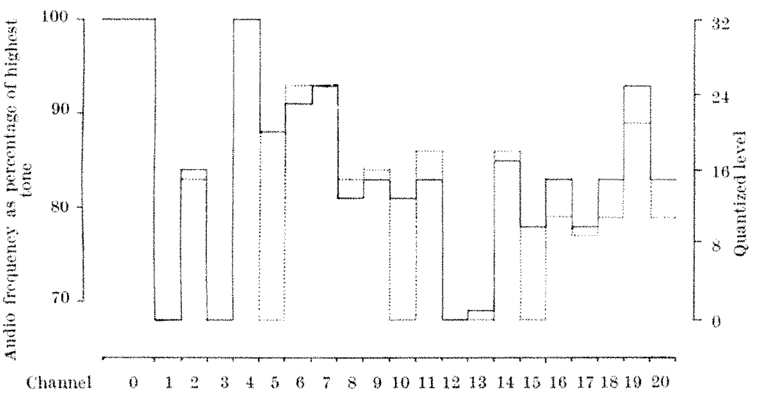

Fig. 2. Telemetry from 1970—34 A on revolution 24 (———), April 26, 1970, and revolution 99 (. . .), May 2, 1970.

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Is the Diffuse Interstellar Absorption Band at 4430 Å caused by Trivalent Iron?

ALTHOUGH the broad diffuse interstellar absorption bands have been known for more than 30 years, they still defy identification. It has been suggested that the bands may be caused by electronic transitions in impurity ions in grains[1]. Here I show that the wavelength, asymmetry and half-width of the 4430 Å band, in particular, are compatible with absorption bands marking the $^6A_1(S)\rightarrow{}^4A_1{}^4E(G)$ transitions in Fe^{3+} in some terrestrial silicates. The absorption spectra of Fe^{3+} in octahedral sites in some garnets are used for comparison, because these spectra are now reasonably well understood. Because Fe^{3+} is a $3d^5$ ion, however, the d–d spectra are similar for both octahedral and tetrahedral complexes.

Fig. 1 shows portions of the absorption spectra of the natural garnets and radite[2], $Ca_3Fe_2^{3+}Si_3O_{12}$, and schorlomite[3], a high-Ti garnet corresponding to an andradite but with approximately 25 per cent of the tetrahedral Si^{4+} and 10 per cent of the octahedral Fe^{3+} ions replaced by Al^{3+} and Ti^{4+} ions[4]. Each oxygen is common to two eight-cornered Ca^{2+} polyhedra, one Fe^{3+} octahedron and one Si^{4+} tetrahedron[5]. In schorlomite, each Fe^{3+} ion has an average of 1·5 Al^{3+} or Ti^{4+} ions in the next-nearest-neighbour tetrahedral sites. The principal bands at 4400 Å mark the $^6A_1(S)\rightarrow{}^4A_1{}^4E(G)$ transitions in Fe^{3+}; they are relatively sharp for transition-metal d–d bands because the transitions are field-independent[2]. The oscillator strengths are $3·5\times10^{-6}$ for andradite and 6×10^{-5} for schorlomite. Substitution of the tetrahedral Si^{4+} ions by the larger, less covalent Al^{3+} or Ti^{4+} ions destroys the inversion symmetry at adjacent octahedral Fe^{3+} sites by distorting the oxygen octahedra and modifying the charge distribution within these octahedra[3]. Accordingly, in schorlomite, the Fe^{3+} transitions may borrow intensity from charge-transfer transitions, such as $O^{2-}2p\rightarrow Fe^{3+}3d$, through crystal field mixing of odd-parity charge-transfer states with even-parity d states. Replacement of Si^{4+} ions by Al^{3+} and Ti^{4+} ions also has the effect of broadening the Fe^{3+} bands; the half-widths of the 4400 Å bands in andradite and schorlomite are 100 Å and 260 Å respectively, compared with a half-width of 150–200 Å for the 4430 Å band in spectra of supernova SN–NGC–2713[6–8].

The $^4A_1(G)$ and $^4E(G)$ levels of Fe^{3+} are not exactly degenerate and in well resolved spectra two component bands are observed (Fig. 1). In less well resolved spectra a shoulder is observed on one of the wings of the 4400 Å band. The image tube observations of Brück and Nandy[9] show that the 4430 Å interstellar band can be highly asymmetric, the longer wavelength side being the steeper. This indicates the presence of a weaker band on the shorter wavelength flank. Hence the asymmetry of the 4430 Å band can be explained by transitions in Fe^{3+}.

The contours of the Fe d–d bands in the silicates are not changed significantly by lowering the temperature to 77 K, although a slight reduction in width occurs. It is conceivable that the further lowering of the temperature would markedly reduce band widths, but the width and oscillator strength of the 4400 Å bands seem to depend more on site distortions and next-nearest-neighbour interactions.

The question of band intensities is of great importance because of the relatively small oscillator strengths of Fe^{3+} d–d bands. With an absorption of $\simeq 0{\cdot}1$ mag. kpc^{-1} at 4430 Å, the density of absorbers[1] is $N \simeq 3 \times 10^{-9}.f^{-1}$ cm^{-3}, where f is the oscillator strength. If, say, $f = 2 \times 10^{-5}$, $N \simeq 1{\cdot}5 \times 10^{-4}$ atoms/cm^3, and for a total grain density of 10^{-11} cm^{-3}, we require $\sim 1{\cdot}5 \times 10^7$ atoms/grain. Thus if each grain has $\sim 10^9$ atoms and if my proposal is correct, interstellar grains contain significant concentrations of Fe^{3+}-bearing silicate.

The Fe^{3+} ions in interstellar dust are likely to be in a silicate matrix because of the position of the ultraviolet absorption edge. In water, for example, charge-transfer bands of hydrolysed Fe^{3+} complexes obliterate the weaker d–d bands. In fact, it is only in the past few years with the study of silicate minerals that the blue region has been sufficiently transparent to resolve weak Fe^{3+} d–d bands. It should be emphasized that the silicate need not necessarily be andradite. Electronic transitions to the ${}^4A_1{}^4E(G)$ levels are observed in the range 4200–4500 Å in the spectra of other silicates also[10], with, in some cases, very different oscillator strengths, depending on the interactions occurring in the crystal structures.

Because Fe^{3+} is a $3d^5$ ion, the energy-level diagram[11,12] is the same for octahedral and tetrahedral complexes. The absorption spectra show similar features[2,13], for example, the ${}^4A_1{}^4E(G)$ levels in Fe^{3+} in tetrahedral sites in phlogopite are also observed at 4400 Å. Unfortunately, d–d spectra of tetrahedrally bonded Fe^{3+} complexes are not yet fully understood. Nevertheless, the energy-level diagram[11,12] shows that the levels ${}^4T_1(G)$, ${}^4T_2(G)$ and ${}^4A_1{}^4E(G)$ are more closely bunched in tetrahedral complexes than in octahedral, because of the weaker crystal field. Bearing in mind that transitions to the ${}^4T_2(G)$ and ${}^4T_1(G)$ levels are observed at ~ 6000 Å and ~ 10000 Å in octahedral complexes[10], it is conceivable that the 4870 Å and 5100 Å bands in interstellar dust[6,7] may mark the same transitions in tetrahedral fields. Significantly, Faye[13] assigns two bands in spectra of phlogopite at ~ 5000 Å to transitions to the ${}^4T_1(G)$ and ${}^4T_2(G)$ levels in tetrahedral Fe^{3+}.

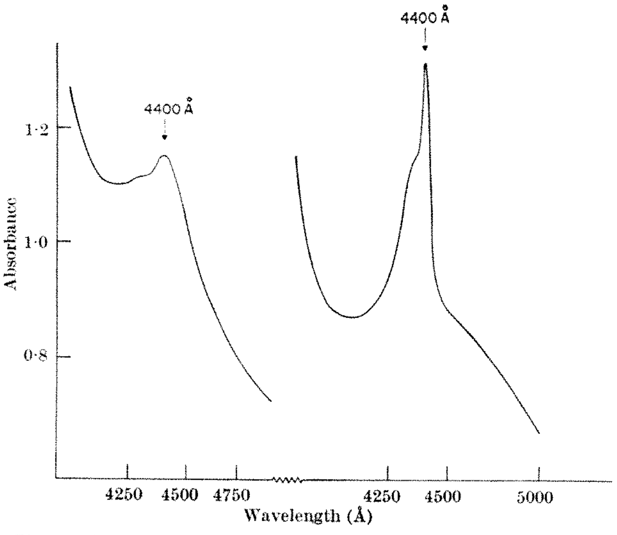

Fig. 1. Absorption spectra of terrestrial schorlomite (left hand spectrum) and andradite (right hand spectrum). Sample thicknesses are respectively 0·0015 cm and 0·03 cm. Temperature = 290 K.

The spin-allowed transition ${}^5T_2 \leftrightarrow {}^5E(D)$ in octahedral or tetrahedral Fe^{2+}–O complexes is generally observed in the near-infrared[2]. The 4430 Å band cannot therefore be assigned to Fe^{2+}.

It seems reasonable to suggest that the 4430 Å interstellar absorption band may be due to the ${}^6A_1 \rightarrow {}^4A_1{}^4E(G)$ transition in Fe^{3+} ions located in a silicate matrix.

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Anomalous Recombination Line 166α

A SPECTRAL line corresponding to the "anomalous" recombination line at 5,011 MHz (109α) described by Palmer *et al.*[1] has been detected at 1,425 MHz (166α) in the HII regions NGC 2024, W3 and possibly W43. The intensity of the anomalous line compared with that of hydrogen is much greater for the 166α line than for the 109α line. A similar enhancement but of smaller magnitude has recently been reported for the anomalous 134α line by Zuckerman and Palmer[2].

The observations were carried out in June 1969 using the Mark I 250 foot telescope (beamwidth 18 min × 12 min arc) in association with the 256 channel autocorrelation spectrometer[3]. The total system noise of the receiver was about 80 K. The spectrometer was used with a 5 MHz bandwidth; the instrumental profile has a $(\sin^2 x)/x^2$ form with a half power width of 34·4 kHz and a 5 per cent secondary response 58·6 kHz from the main response. The receiver was used in a frequency switching mode in which the signal was compared with frequency bands displaced $\pm 1{\cdot}3$ MHz. The resulting spectra had curved baselines with maximum slopes of about 0·5 K MHz^{-1}. These were removed by subtracting a fourth order polynomial which had been fitted to the parts of the spectra adjacent to the recombination lines.

The resulting spectra from NGC 2024, W3 and W43 are shown in Fig. 1. It has been suggested by Goldberg and Dupree[4] that the element emitting the anomalous line is carbon and hence the frequencies of the helium and carbon lines (1,425·314 MHz and 1,425·445 MHz) are marked on the figure relative to the frequency of the hydrogen recombination line (1,424·734 MHz). For NGC 2024 and W43 the line does not lie exactly on the frequency of the carbon line, but this could be accounted for by the carbon having a different velocity from the hydrogen. The line temperature of the anomalous line in NGC 2024 is about 50 per cent of the hydrogen line temperature and may be even larger if the line is not resolved in frequency. By comparison the anomalous 109α line temperature, given by Palmer *et al.*, is about 10 per cent of the hydrogen 109α line temperature. The helium 166α line is $\lesssim 6$ per cent of the hydrogen line intensity in NGC 2024 and is consistent with the results

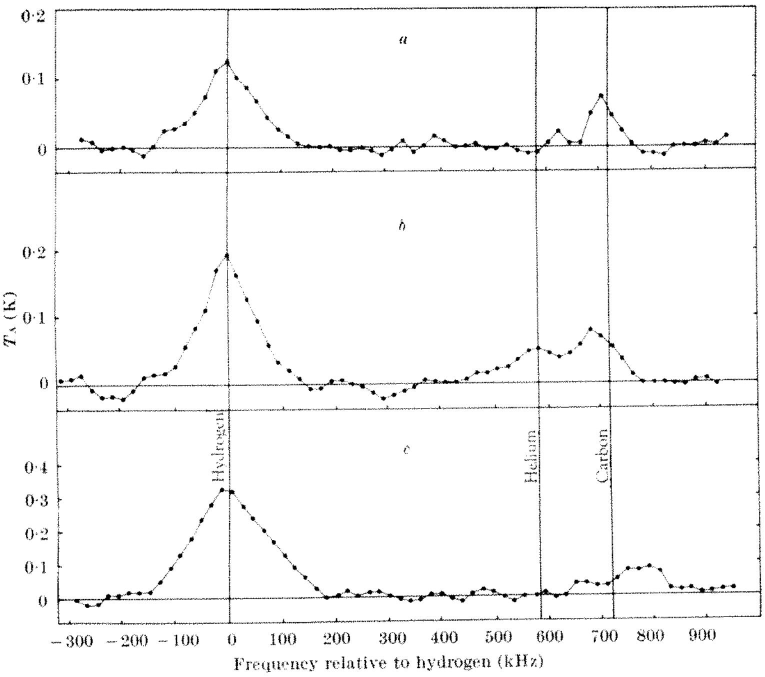

Fig. 1. Spectra of NGC 2024 (*a*), W3 (*b*), and W43 (*c*) in the vicinity of the frequency of the hydrogen 166α transition (1,424·734 MHz). The frequencies of the carbon 166α and the helium 166α are shown assuming that they have the same velocity as the centroid of the hydrogen profile. The velocities of the hydrogen centroids, relative to the local standard of rest ($V_{l.s.r.}$), are: *a*, +7·6 km/s; *b*, −40·1 km/s; *c*, +99·4 km/s.

of Palmer *et al.* for the 109α line. An estimate of the width of the anomalous lines can be made from Fig. 1, although the relatively wide spectrometer resolution makes the results somewhat inaccurate. The half power width for NGC 2024 is 43^{+20}_{-30} kHz after instrumental broadening has been removed. It is difficult to estimate the width of W3 on account of confusion with helium.

Table 1. LOWER LIMITS ON SEVEN SOURCES

	Source	R.A. (1950) h m s	Dec. (1950)	Int. time (min)	E_L/E_H (166α)	E_L/E_H (134α)	E_L/E_H (109α)
W12	NGC 2024	05 39 10	−01° 05′	315	0·35 ± 05	0·064	0·030
W3	IC 1795	02 21 39	+61 53	360	0·20 ± 0·05	0·037	0·028
W43		18 44 42	−02 06	150	0·08 ± 0·02	—	—
	DR 23	20 39 05	+41 46	105	< 0·17	—	—
W37	M16	18 16 05	−13 52	90	< 0·16	—	—
W37	M16	18 15 40	−13 44	120	< 0·10	—	—
W35		18 15 00	−12 00	75	< 0·13	—	—
W49		19 07 48	+09 01	105	< 0·10	—	—
W29	M8	18 00 34	−24 22	90	< 0·07	—	—
W38	M17	18 17 33	−16 12	90	< 0·03	—	—

The errors quoted for E_L/E_H for W12, W3 and W43 are r.m.s. values.

Lower limits on seven other sources are given in Table 1. These are essentially a measure of the peak to peak error, and are given in units of E_L/E_H, the ratio of the energy in the anomalous line to the energy in the hydrogen line. The last two columns show the values of E_L/E_H for the 109α lines[1] and the 134α lines[2].

E_L/E_H varies considerably between the sources. The values of NGC 2024 and W3 are 0·2 to 0·35, whereas at the other extreme the ratio is < 0·07 for M8 and M17. This indicates that the emission is strongly dependent upon the parameters of the HII regions themselves or of the interstellar medium lying in front of the HII regions[5]. It is not yet clear from the data available, however, what parameters are significant in producing the enhancement of the anomalous line relative to hydrogen.

Another interesting point is the variation of E_L/E_H with frequency. For the 109α line E_L/E_H is only 3 per cent for NGC 2024, whereas for the 134α line it is 6·4 per cent and for the 166α line E_L/E_H is at least 30 per cent. This definitely shows that the line is not due to an abundance anomaly, for the ratio would be independent of the *n* quantum number of the states involved. Similarly, it is improbable that both the hydrogen and the anomalous line are emitted from HI regions as suggested by Greenberg[6], because again one would not expect E_L/E_H to vary with *n* value. The smaller widths of the anomalous lines relative to the hydrogen lines as shown in Fig. 1 and previous observations[1,2] also indicate[5] that both the hydrogen and anomalous lines do not arise from an HI region.

It seems unlikely that the invocation of dielectronic recombination, already in difficulties in explaining a factor of ~60 for the 109α line, can produce overpopulations of ~600 for the 166α line (assuming $N_{\text{carbon}} = 5 \times 10^{-4} N_H$) unless unreasonably high temperatures and low electron densities are assumed[7,8].

The results are not in conflict with the theory of Dupree and Goldberg[5] nor that of Zuckerman and Palmer[9]. The former proposes that emission from the upper states of carbon atoms in HI regions, adjacent to HII regions, is enhanced by stimulated emission caused by the continuum radio emission from the HII region. The latter theory proposes that the emission occurs in cool, high-density HI regions within the HII regions. These are optically thick to 13·6 eV ultraviolet radiation and hence only elements with ionization potentials less than this, for example carbon with 11·3 eV, will be ionized. Before the compatibility of these theories with the observations can be checked, a knowledge of the behaviour of the b_n factors for carbon at temperatures ~ 100 K is required. These are as yet not available.

The width of the anomalous line in NGC 2024 at 5,011 MHz is 64 kHz as measured by Palmer *et al.* and hence at 1,420 MHz one would expect the width to be 18 kHz. The observed width is 47^{+20}_{-30} kHz, and hence because of the errors it is impossible to state, on the basis of the present observations, that the line width is definitely deviating from Doppler broadening. The results of Zuckerman and Palmer[2] suggest that the 134α line may be broadened, but again the errors are rather large. If such a deviation existed it might be the result of "Stark" broadening[10] occurring in the type of high density region proposed by Zuckerman and Palmer[9].

The observations show that the population of the upper states of the element emitting the anomalous line is far from thermodynamic equilibrium, whether it is emitted in the same or a different region from the hydrogen. To obtain more information on these phenomena it will be of interest to observe these anomalous lines at larger *n* numbers.

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Temperature Microstructure in a Fresh Water Thermocline

RECENT measurements of microstructure in the ocean[1-4] have led to considerable speculation about physical processes that lead to a steplike distribution of temperature, salinity and velocity. Briefly, one school of thought explains the microstructure in terms of interleaving of layers originating at different locations[1,2]. The other school suggests that the transport processes responsible for the vertical diffusion of heat, salt and momentum may inevitably result in the development of layers; the most striking theory, the so-called salt finger mechanism developed by Turner, Stommel and Stern[5-7], depends on the difference between the molecular diffusivities for salt and heat. This mechanism works only in a statically stable ocean where the temperature gradient is stable, but the salinity gradient is unstable, or in the reverse situation. Tait and Howe[3] have recently published profiles that strongly favour the salt-fingering process for layers under the Mediterranean outflow, but regular steps in temperature and salt of this kind are rare: the great majority of ocean microstructure is much more variable. Dr R. W. Stewart (private communication) has suggested that this microstructure is caused by the difference between the (turbulent) transport coefficients for buoyancy and momentum. No adequate theory for this buoyancy–momentum process has yet been developed, but the low Reynolds number turbulent spots discovered by Woods[8,9] may be important. Temperature microstructure in a freshwater lake at least demonstrates that salinity gradients are not an essential factor in the generation of ocean microstructure. The occurrence of such microstructure in freshwater lakes seems likely from transparency observations made by Whitney[11].

Preliminary observations in Lake Bala in Merionethshire[12] gave positive indications of microstructure, and we decided to make further observations of the seasonal thermocline of Loch Ness in September 1968. This Scottish freshwater lake is about 30 km long and 1 km wide and has an average depth of nearly 200 m.

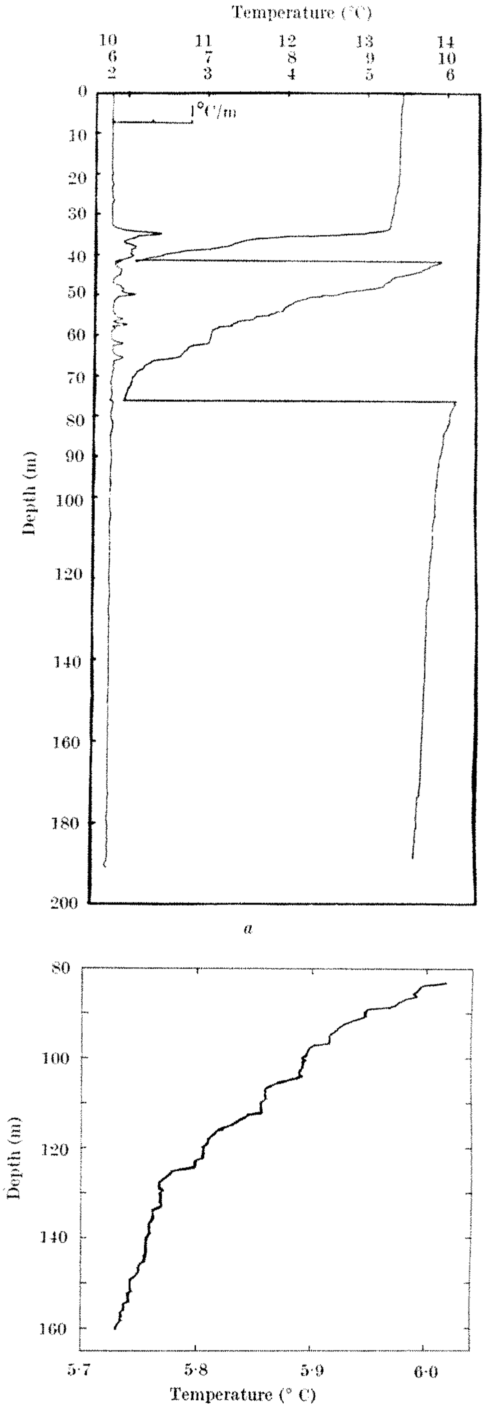
Temperature and temperature gradient profiles were measured with a thermocline microstructure probe[10] and a bathysonde system operated from the RV Prince Madog. In calm weather the microstructure probe is capable of better than 2 cm vertical resolution. The bathysonde has lower resolution (~40 cm) but can make soundings to the full depth of the lake and is less seriously affected by ship movement in adverse weather conditions.

The bathysonde sounding shown in Fig. 1*a* illustrates the gross thermal structure of the lake. There is a mixed layer approximately 30 m thick at the surface below which the seasonal thermocline extends down to about 100 m. A rich microstructure is evident on the thermocline with alternating regions of intense and weak temperature gradient (sheets and layers).

Below 80 m, the mean temperature gradient is small but fine structure is still present. A series of almost isothermal layers about 5 m thick can be seen in Fig. 1*b*.

The thermocline features can be seen in more detail in the microstructure probe soundings (Fig. 2*a*). The profile in Fig. 2*b* was taken with the same apparatus in the Mediterranean, 20 km south of Malta. There is a marked similarity between the form and spacing of the microstructure in these two profiles. The principal difference is the existence of negative temperature gradients (inversions) in the Mediterranean profile. Such features would be unstable in freshwater, but commonly occur in the ocean where stability may be maintained by salinity variations. Dr C. S. Cox (unpublished manuscript of Scripps Institution of Oceanography) has made similar measurements at a depth of 400–600 m in the Pacific using a slowly falling probe with a resolution of 1 cm. The spectral characteristics of the vertical scale of this deep microstructure are essentially the same as those found at Loch Ness and near Malta.

Fig. 3 shows a sequence of eleven temperature profiles observed at 15 min intervals. The detail of the profiles varies, but certain step features (for example, at about 11·1° C) persist throughout the sequence indicating a time scale of hours at least. Observations of the persistence of similar large step features in the ocean have



a

b

Fig. 1. *a*, Temperature profile (on the right) and temperature-gradient profile (on the left) at Loch Ness station 9 on September 21, 1968. Both profiles have been drawn from digital data spaced at intervals of 20 cm, obtained from the bathysonde. *b*, An expanded scale plot of the temperature in the bottom layer showing a number of almost isothermal layers, differing in temperature by only a few hundredths of a degree Celsius. Temperature resolution is limited by the digital system of recording to ±0·001° C.

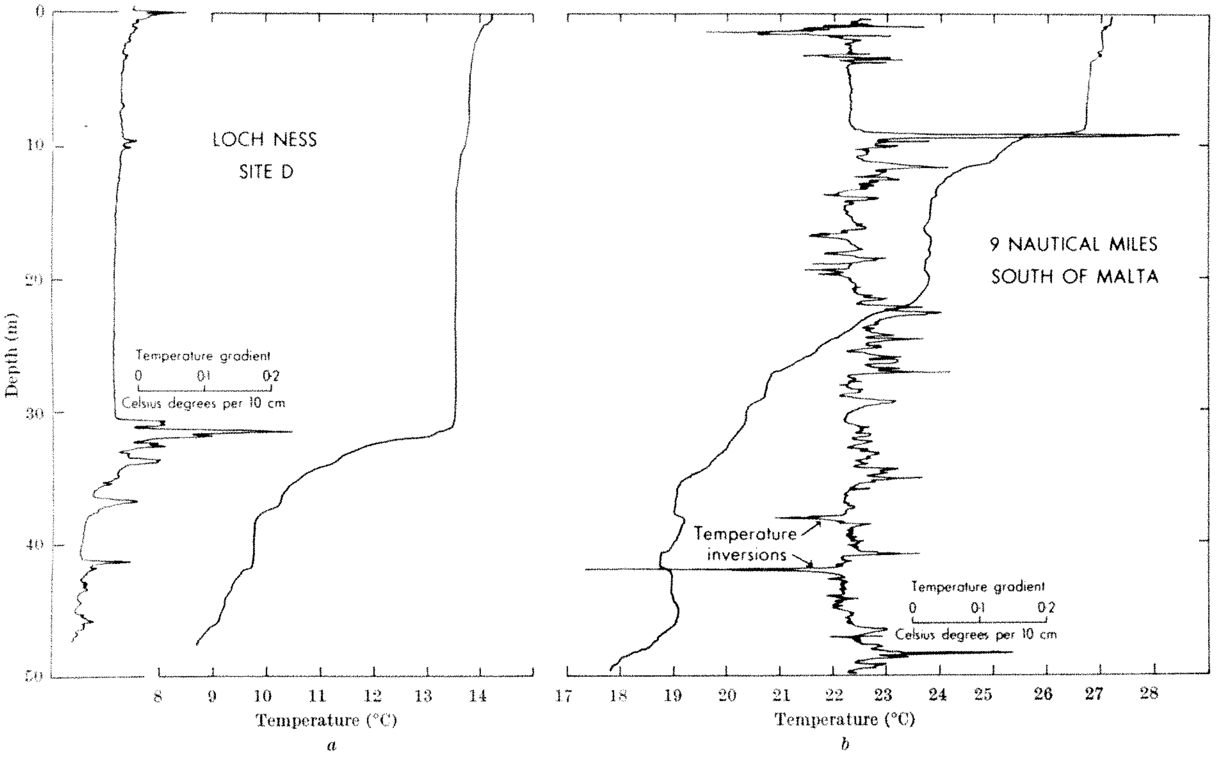


Fig. 2. *a*, A temperature and temperature-gradient sounding through the seasonal thermocline in Loch Ness, September 19, 1968. The temperature-gradient profile represents the temperature difference across two platinum resistance thermometers fixed 10 cm apart; the temperature profile is obtained from a third thermometer and the depth for a pressure transducer. (There is a slight instrumental drift with depth in the temperature-gradient trace, which is also displaced upward by approximately one metre and has arbitrary zero.) *b*, A temperature and temperature-gradient sounding through the seasonal thermocline of Malta, August 1968, made with the same microstructure probe as was used in the Loch Ness expedition. The profile is plotted to a vertical resolution of 10 cm; the sensors have a somewhat better resolution.

recently been reported by Cooper and Stommel[4]. The apparent lack of persistence of some of the smaller features in Fig. 3 may be a result of mixing caused by the protective framework surrounding the bathysonde temperature sensor; successive soundings made at 30 min intervals with the microstructure probe did show persistence in these fine details.

We were unable, during our short stay in the loch, to measure satisfactorily the spatial extent of individual features of the fine structure. We did, however, find in the loch an interaction between the horizontal layering and horizontal temperature gradient. During our stay in Loch Ness, the temperature of the mixed layer at the south-west end of the loch was almost exactly 1° C lower on the north-west side than on the south-east side. Our measurements made between Fort Augustus and a point midway between Invermoriston and Foyers suggest that a sharp 1° C "front" runs parallel to the (SW–NE) axis of the loch. Fig. 4 shows three microstructure soundings at positions *A*, *B*, *C* on the map. A comparison of the microstructure at the warm water sites, *A* and *B*, shows that the general shape of the temperature profile is similar, and that many of the temperature-gradient spikes (sheets) occur at the same temperature (although their depths do not necessarily correspond, for the isotherms are tilted by internal seiches and waves) and have a similar form in each profile. At the cold water site *C*, the broad thermocline profile is more stepped and the sheets do not occur at the same temperature as at *A* and *B*. This method of sheet-by-sheet comparison contains an element of subjective assessment, but a satisfactory objective test for the coherence of individual features in successive profiles has not yet been devised. The method shows, however, that many of the sheets in Loch Ness extend at least 1/2 km horizontally when the surface temperature gradient is small, but that a 1° C "front" acts as a barrier to the microstructure. This conclusion has since been shown to hold for the Maltese seasonal thermocline, details of which will be published later. A similar comparison between the profiles at sites *A* and *B* and another warm site *D* shows that the strongest sheets (those with the greatest temperature difference) extend at least as far as 1·5 km.

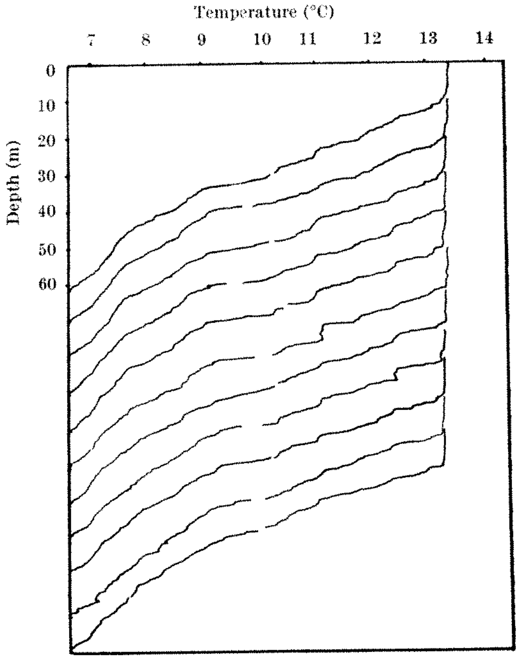


Fig. 3. Repeated temperature profiles at Loch Ness station 12B, September 20, 1968, at 15 min intervals. Each sounding has been offset by 10 m from the preceding one. The short break in the trace is caused by a change of range on the bathysonde.

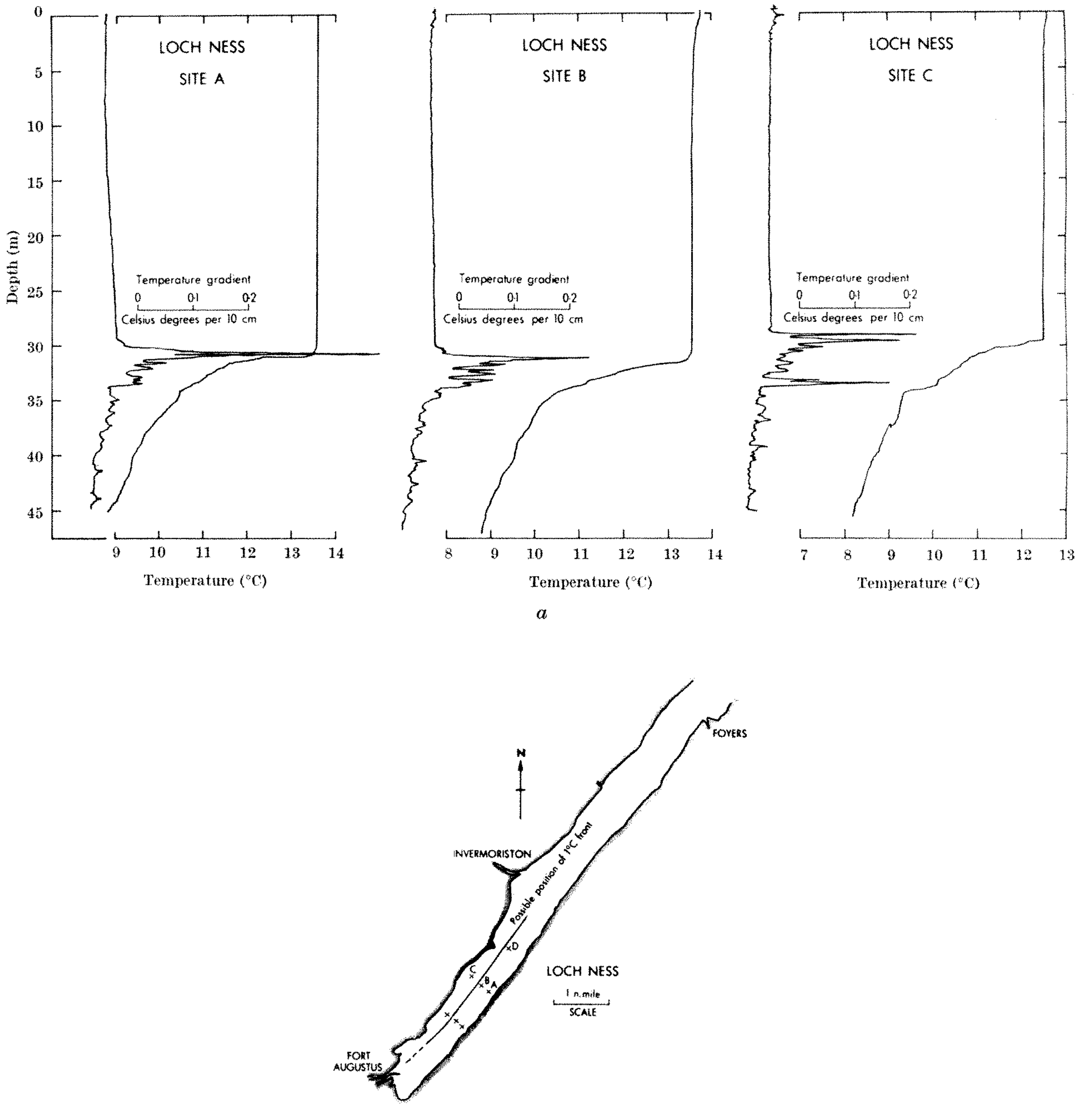


Fig. 4. *a*, Temperature microstructure at positions *A* and *B* in the warm water and position *C* in the cold water. The temperature sheets in *A* and *B* do not penetrate the front and an independent microstructure is found on the other side at *C*. *b*, Approximate position of the "front" at the Fort Augustus end of Loch Ness.

The existence of such a microstructure, in the absence of any dissolved or suspended matter, suggests that salt does not necessarily play an essential part in the formation of microstructure on the oceanic thermocline. The vertical scale of the thermocline microstructure found in Loch Ness is indistinguishable from that found in the Mediterranean thermocline and in weakly stratified water in the Pacific Ocean. Most of the fine structure detected in Cox's[14] probe extends for at least 50 m, while the coarser features detected by Cooper and Stommel[4] seem to extend for several kilometres. The temperature differences across the sheets in Loch Ness thermocline profiles are about 20 times greater than those in Cox's Pacific profiles. The sheets' horizontal extent is correlated with their relative, rather than absolute, static stability. If, as we suspect, the layering depends on the form of the turbulent spots found off Malta, then it is likely that the correct parameter for classifying the sheets is their gradient Richardson number[13] because this controls the turbulence. A suitable probe for measuring the local gradient Richardson number to a vertical resolution of 10 cm has, however, yet to be developed.

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Variable Boundary, Two Dimensional Tidal Model

THE application of digital computers to tidal problems has revived interest in tides, including the problem of the numerical tidal modelling of estuaries or bays in one or two dimensions. Usually computer programs have been designed to find a solution for one tidal constituent, the M_2 tide, and are applicable only to specific estuarine systems. We report here a two dimensional program which can easily be used to solve semidiurnal tidal problems for many estuaries.

The mathematical basis for the analysis is derived from the two dimensional explicit model originally developed[1] for the North Sea. Other authors[2,3] have given the full mathematical derivations and only the basic differential equations will be given here. The equations of motion in two horizontal dimensions and the equation of continuity are used as follows:

$$U_t + r(U^2 + V^2)^{1/2}\frac{U}{H} + fV + gZ_x = 0$$

$$V_t + r(U^2 + V^2)^{1/2}\frac{U}{H} - fu + gZ_y = 0$$

$$(HU)_x + (HV)_y + Z_t = 0$$

where U is the x component of depth-mean velocity, V the y component of depth-mean velocity, Z the tidal elevation above equilibrium depth, r the friction coefficient, f the Coriolis parameter, H the total water depth and g the gravitational acceleration. Atmospheric and convectional influences are neglected and the water is assumed to be homogeneous.

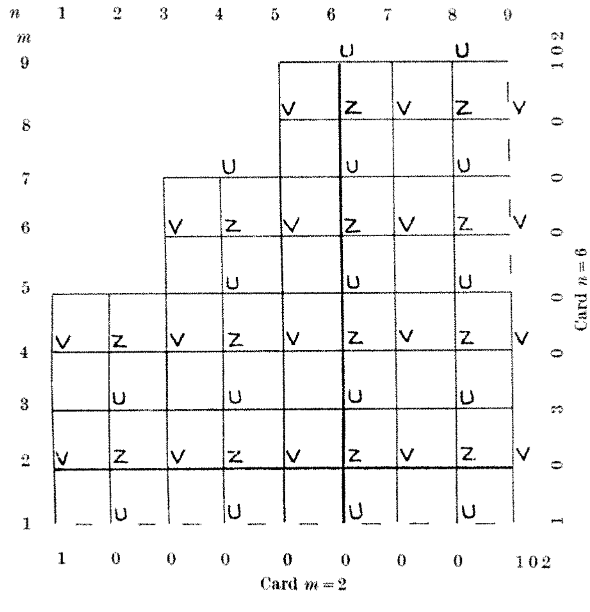

Fig. 1. Part of the two dimensional grid; typical column and row through Z-point, with associated matrix and input cards.

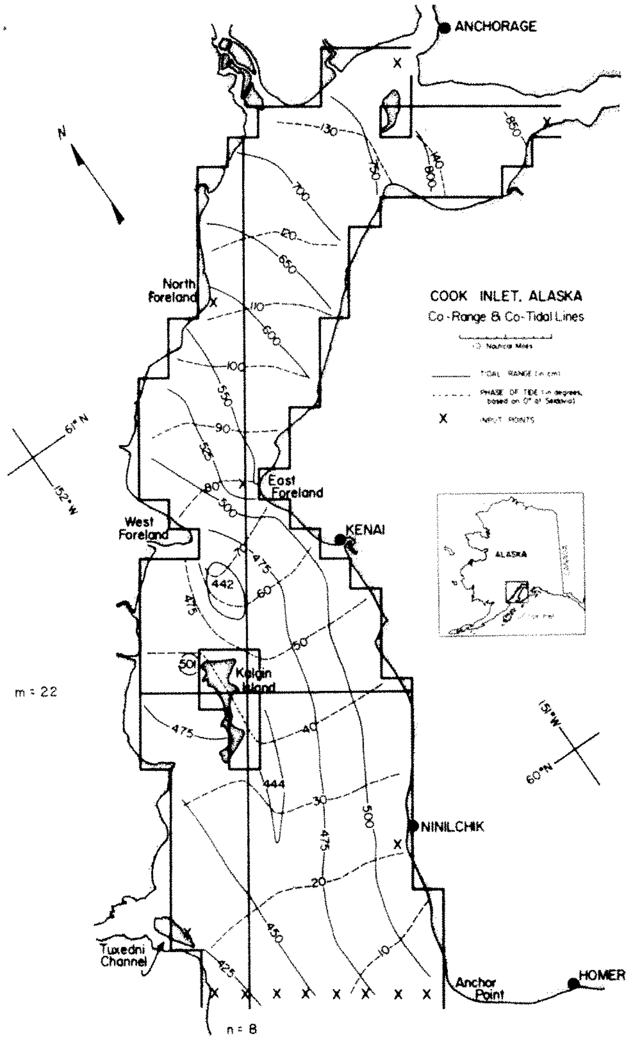

Fig. 2. The grid chosen for Cook Inlet, Alaska, and the predicted co-range and co-tidal lines.

Solution of the equations, by finite difference methods, is facilitated by the grid layout (Fig. 1) and is staggered in time and space. The grid can be terminated at alternate lines in both directions to represent either open or closed boundaries. The only requirements for the grid are that the boundaries in the x direction be terminated by lines of U velocity points and those in the y direction by V velocity points. Within these requirements any shape of boundary can be represented. In practice the boundaries are chosen to coincide with the coastlines of the estuary that is being modelled.

The most novel and useful feature of our model is the way in which the gridshape is specified. Separate specifications are made for horizontal and vertical grid lines, giving the immediate advantage of greatly reducing the number of special calculations to be made in corners of the grid[4]. This is achieved by using an integer matrix.

The integer control matrix is built in the computer from separate linear integer matrices for each row and column of the grid. In our program the input is by punched card, and typical cards for row $m = 2$ and column $n = 6$ are shown in Fig. 1. The V and Z points and the U and Z points lie in the even-numbered rows and columns respectively. Only these rows and columns need be specified in building a matrix. For example, consider $m = 2$ in Fig. 1.

The digit 1 signifies land or a sea area needing no computation. It is placed in the odd-numbered boundary column. Further 1s designate other boundaries and must occur in pairs in any row or column. For a sea/sea boundary a digit 3 is placed in the odd-numbered column to the calculation side of the boundary. To avoid ambiguity, at least one blank odd-numbered column must lie between any code digits 3. A code digit 2 in the next odd column after the boundary terminates the matrix.

The integer matrix controls the program operation. No computations are carried out for points outside a pair of 1s, nor beyond the 1 followed by a 2. At boundary points with 1s, V or U is set to zero for rows or columns respectively except when a digit 3 refers to that point, in which case

$$\frac{\mathrm{d}V}{\mathrm{d}y} \text{ or } \frac{\mathrm{d}U}{\mathrm{d}x}$$

is set to zero instead. The master integer matrix thus controls the program operation. It supervises the allocation of depths, the computation of currents and tidal heights and the calculation of mean range, phase of tide, current ellipses and other parameters. For application of the program to a new inlet, only the integer matrix need be changed and new input data read into the computer.

The model has been applied to several examples, both theoretical and practical, with excellent results. The co-range and co-tidal lines predicted by the model for Cook Inlet are shown in Fig. 2. The results are for a semi-diurnal tide of period 12·42 h and amplitude of one half the mean range. The greatest error in the range was 8 per cent in the vicinity of Kenai.

We think the model should find many applications because of its versatility and simplicity. It is written in Fortran IV with some sub-routines in machine language for the IBM360/40. It is readily adaptable to other machines capable of handling Fortran IV language. Once the program is available it takes only 1 or 2 days' work to make a new master matrix starting from the charts of the area under investigation.

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Lithification of Deep Sea Sediments by Pyrite

UNIQUE pyrite-cemented silty sand layers have been found in three piston cores taken from the North Carolina lower continental rise at depths from 4,770 to 4,950 m (Fig. 1). The layers are approximately 0·5 cm thick and consist mainly of terrigenous grains in a cement identified as pyrite by X-ray diffraction analysis. None of the precursor iron sulphides commonly associated with sedimentary pyrite[1] were found. Single layers at core depths of 530 and 870 cm were noted in cores *B* and *C* respectively (Fig. 1). In core *A*, two pyritized silty sand layers occur at depths in the sediment of 540 and 560 cm. Fig. 2 is a photograph of a fragment of one of the lithified sand layers. The rock has approximately the same 6 cm diameter as the piston core cutter, suggesting strongly that the rock was punched out of a pyritized sheet of unknown lateral extent by the core tube.

Thin sections of the rocks reveal the presence of individual euhedral cubic pyrite crystals as well as the relative abundance of the framework grains. Calcareous grains incorporated into the rocks seem to have undergone selective dissolution during cementation. Outer surfaces of the two foraminifera tests in Fig. 3 exhibit

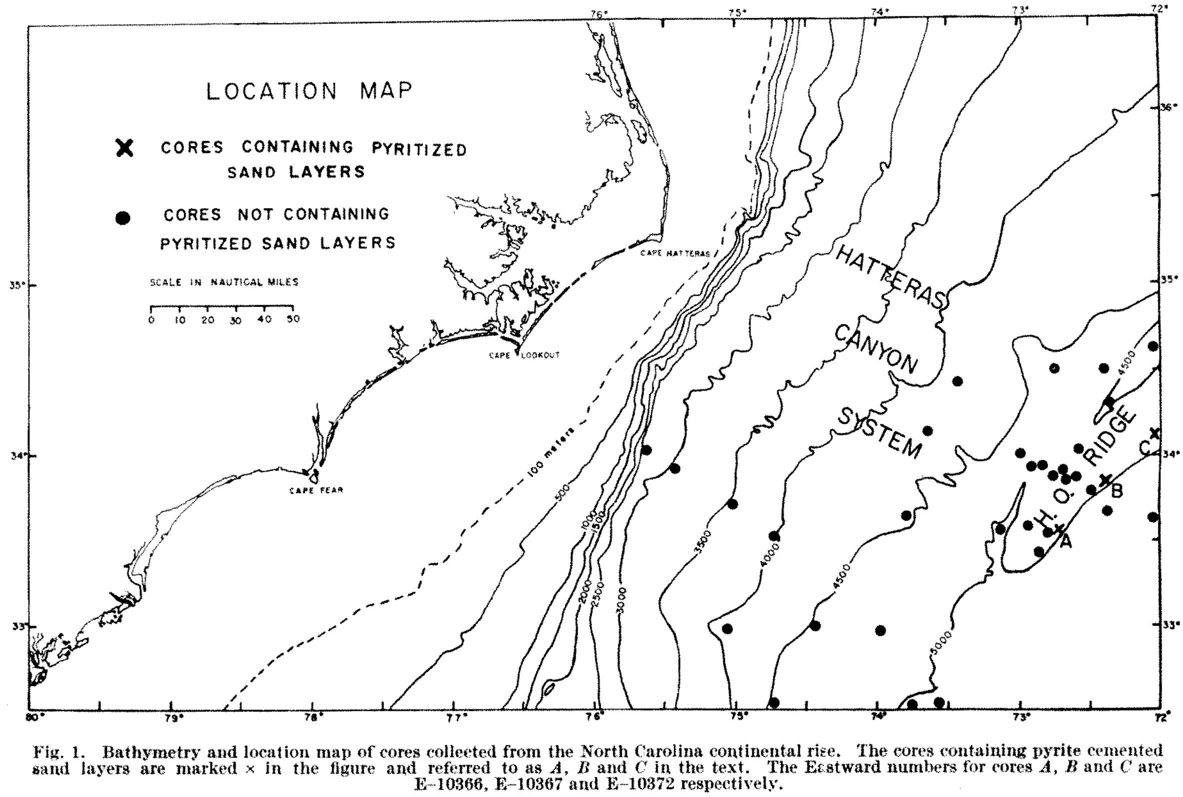

Fig. 1. Bathymetry and location map of cores collected from the North Carolina continental rise. The cores containing pyrite cemented sand layers are marked × in the figure and referred to as *A*, *B* and *C* in the text. The Eastward numbers for cores *A*, *B* and *C* are E-10366, E-10367 and E-10372 respectively.

different stages of partial dissolution and subsequent replacement by pyrite. The few calcareous particles present in the pyritized sandstones are relatively coarse, and in view of the strong evidence of solution effects on these grains it is probable that finer calcareous material has already been selectively removed. Extensive solution of $CaCO_3$ is to be expected during authigenic formation of pyrite[2,3].

Of the thirty-five piston cores collected as part of a continuing study of the North Carolina continental rise, only three contain pyritized sandstone. All three are from the seaward flank of the Hatteras Outer Ridge (Fig. 1) and we propose that formation of the pyritized sand layers is related to the unique environmental conditions on the seaward flank.

Sediment is probably delivered to the outer ridge by both turbidity currents and southerly flowing bottom "contour" currents[4]. The turbidity flows emanate from the Hatteras Canyon System along paths controlled primarily by the Hatteras Outer Ridge[5]. A few turbidity currents cross the 400 m high ridge and deposit material on the seaward flank; most, however, are probably blocked or deflected by the ridge[5]. The ridge blocks most high velocity canyon flows by preventing them from reaching the seaward flank and thereby creating an overall low rate of sedimentation.

The few turbidity currents that cross the ridge carry both sands containing fauna from the continental shelf edge and fine grained, typically organic enriched[6], sediment from the continental slope[1,5]. Displacement of slope and shelf sediments to the outer ridge provides the relatively organic-rich fine grained sediment that generates sulphide as well as the clean sands which constitute a trap for the migrating sulphide. The unusual combination of typically organic-rich continental margin sediment deposited in rapid pulses but accumulating at overall rates slower than usual may be the important causative factors for extensive pyritization in the uppermost portions of the sediment column. The pyritized layers are also geologically significant because they must play a part in restricting and channelling vertical exchange between seawater and sediment as well as in interstitial water reactions and migrations. Although the layers of pyritized sandstones are thin and volumetrically unimportant in the local sediment column, the occurrence is of great interest because such cementation by pyritization is only the second known mechanism of lithification in the uppermost portions of deep sea sediments. Previously known deep sea lithification at shallow sediment depths[7] has involved cementation by $CaCO_3$.

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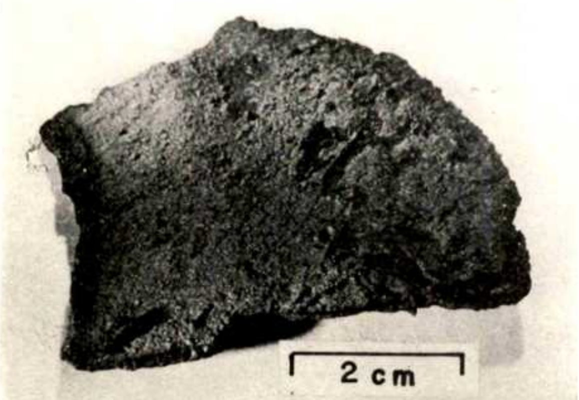

Fig. 2. Pyrite cemented sand layer taken from core *A* (E–10366) at a sediment depth of 540 cm. The uneven shape was caused by irregular fracturing during penetration.

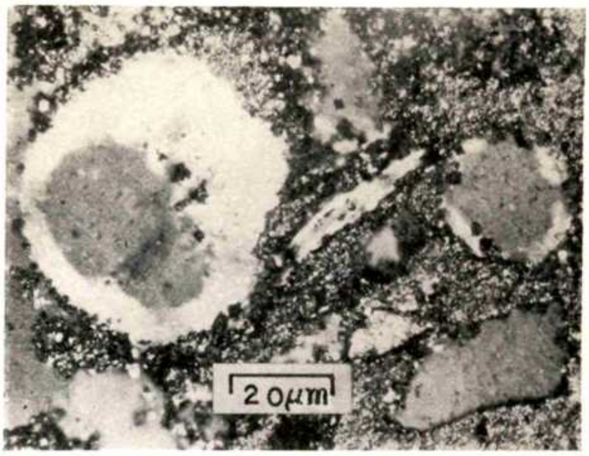

Fig. 3. Photomicrograph of thin section from the rock found in core *C* (E–10372) at a sediment depth of 870 cm, showing two partially dissolved planktonic foraminifera in pyrite cement. The right foraminifera shows more advanced stage of solution than the left.

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Long-lived Thermal Structure under the Southern Rocky Mountains

HOT regions in the upper mantle can be located in two ways. The first is the classical technique of mapping heat flow through the crust from measurements at numerous points. The more recent method of geomagnetic deep sounding (GDS) uses recording magnetometers at a number of stations to locate regions of enhanced electrical conductivity in the Earth through the magnetic fields of transient currents. Some, at least, of the ultrabasic silicates of the mantle are semiconductors which show a sharp rise of conductivity[1] at temperatures above 1,000° C. While there is no one-to-one correspondence, it is probable that many of the known upper-mantle conductivity anomalies result from high temperatures. GDS is therefore an important means of seeking hot regions, hitherto limited by the small numbers of magnetometers available. Ocean floor spreading and plate tectonics have revolutionized earth science in the past few years. GDS affords means of extending study of the dynamic mantle demanded, especially under continents where little is yet known.

During 1967 we operated a roughly rectangular array of 42 three-component magnetometers[2] between latitudes

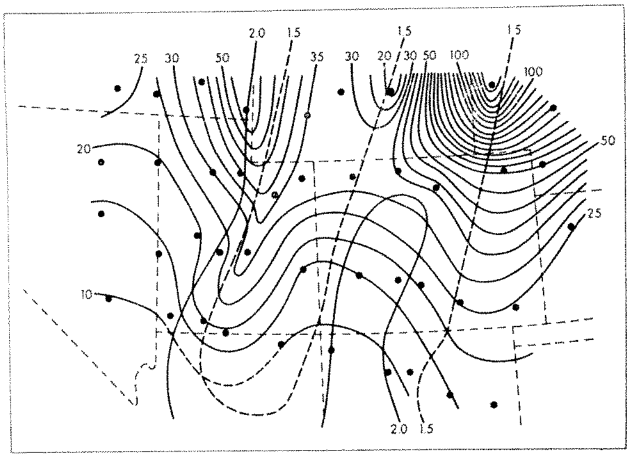

Fig. 1. Heat-flow isolines at 1·5 and 2·0 μcalories/cm^2 s from a provisional map by Roy, Blackwell and Decker (reproduced with their permission) with contours of Fourier spectral amplitude at $T = 60$ min of the vertical field Z of a substorm on September 1, 1967. Unit for Z is 0·1γ. Note tracking of high Z along the east flank of each heat-flow high, corresponding to currents under the heat-flow maxima. Dots represent variometers, straight broken lines outline Utah, Colorado and neighbouring states.

36° and 43° N and longitudes 101° and 116° W. The array locates transient electric currents within the Earth induced by external ionospheric currents during magnetic disturbances, and allows separation of the variation fields into parts associated with the external and internal currents. The analysis of magnetic fields of a polar substorm and of a storm, recorded during September 1967, has revealed two anomalies associated with transient electric currents in north–south conductors beneath the Southern Rockies in Colorado and beneath the Wasatch fault belt in Utah. The latter conductive structure seems to be continuous with that discovered by Schmucker[3] in southern New Mexico and the former is that discovered by Reitzel[4] in Colorado. The induction anomalies, identified by mapping amplitudes and phases of spectral components of the variation fields[5], are closely related to the pattern of heat flow through the crust of the Earth (personal communication from R. F. Roy, D. D. Blackwell and E. R. Decker) (Fig. 1). By using the array to separate the internal fields we have been able to propose[6] a model of highly conducting structures beneath this part of the United States to account for the induction anomalies (Fig. 2). While such models cannot be unique, ours has the advantage that the conductivity required (2×10^{-12} e.m.u.) is reasonable[7] for upper mantle material at 1,500° C, a temperature which is acceptable[8] at the 360 km depth of our conductor away from the anomalous ridges on its surface.

The GDS study is fully reported elsewhere[5,6]. Our purpose here is to show that the coincidence of the GDS and heat-flow anomalies means not only that the conductive structures related to the former are almost certainly thermal, but also that these parts of the upper mantle have been hot for a long time. Low thermal conductivities in the upper mantle and crust delay thermal information from depths of order 100 km for times of the order of 10^8 yr. A structure in the upper mantle responsible for both heat-flow and electromagnetic induction anomalies must have persisted for a time of this order, or else must have reappeared. The coincidence of heat-flow and induction anomalies greatly increases the probability that associated upper-mantle structures are old enough to be connected with the origins of overlying mountains and structural provinces.

Let t_h be the time required for a heat-flow anomaly to appear at the Earth's surface after a source appears in the upper mantle. As source one could, using numerical integration, take the whole surface S of the two-dimensional model whose cross-section is shaded in Fig. 2. Such a computation would be justified only if one knew more of the thermal conductivity in the upper mantle. We take a line source at the minimum depth of S; this gives a lower bound to t_h. Suppose a horizontal line source of heat appears at $t = 0$ at depth D in a semi-infinite medium, initially at zero temperature, of thermal diffusivity $\varkappa$ and whose horizontal plane surface is kept at temperature $\theta = 0$. If the line source produces heat at constant rate for all positive t, it can be shown by the method of images[9] that θ at depth d vertically above the line source rises as

$$\frac{\theta}{\theta_\infty} = \left[2 \ln\left(\frac{D-d}{D+d}\right)\right]^{-1} \left\{ Ei\left[-\frac{(D-d)^2}{4\varkappa t}\right] - Ei\left[-\frac{(D+d)^2}{4\varkappa t}\right]\right\} \qquad (1)$$

where $-Ei(-x) \equiv \int_x^\infty \frac{e^{-u}}{u}\,\mathrm{d}u$ and θ_∞ is the temperature after a very long time. With $\theta = 0$ at $d = 0$, heat flow through the surface will grow as θ at small d.

Of the two conductive ridges of Fig. 2 we choose that under the Southern Rockies, because the conductor and the heat-flow anomaly are symmetrical and because recent vulcanism complicates interpretation of the heat flow in Utah but not of that in the Southern Rockies. For D we take 210 km, the depth to the top of the conductive structure, and for $\varkappa$ 0·04 cm^2/s. The latter is too large for lattice conduction and is adopted to make allowance for possible partial melting; radiative transfer is probably unimportant below 1,500° C. The growth of the heat-flow anomaly due to the line source is shown in Table 1: that due to the real structure will grow more slowly. To estimate t_h one needs an estimate of the maturity of the anomaly, θ/θ_∞. If the surface of the conductor in Fig. 2 is an isotherm, the thickness of the overlying material is 210 km under the Southern Rockies and 360 km either side. The heat flow in the steady state should be somewhat less (because of the geometry) than 360/210, or 1·7 times greater under the mountains than

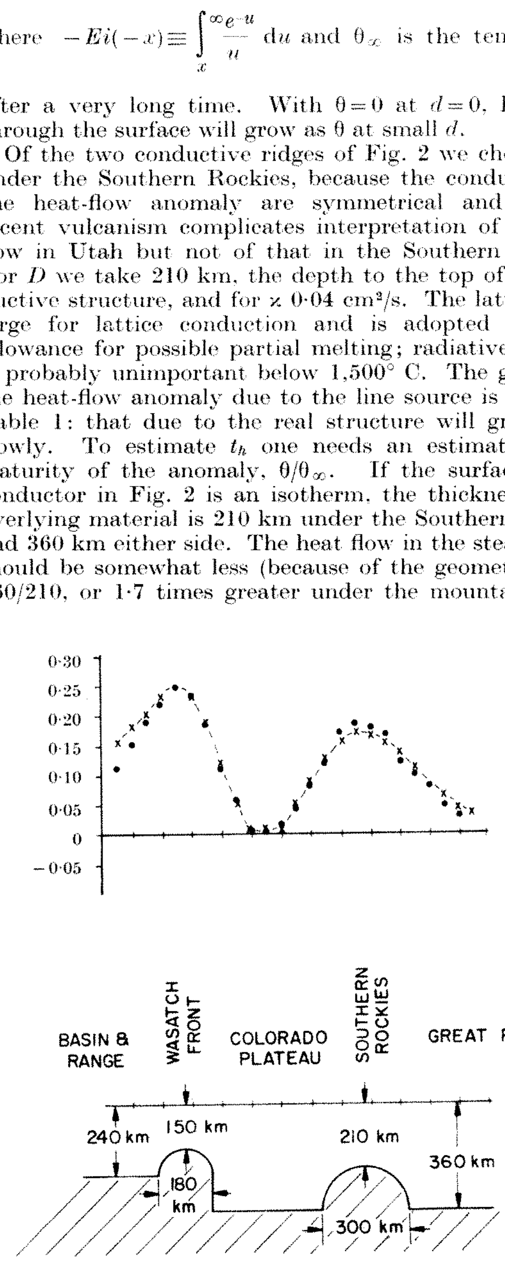

Fig. 2. Conductivity model approximating the eastward horizontal internal anomaly field Y_{ia}. The ordinate in the upper figure gives Y_{ia} expressed as a fraction of the east–west field which induces currents in the conductive structures. ×, Observed; ●, model.

in the plains. After correction for radioactive heat sources in the crust, the heat-flow data suggest a ratio[10] of about 1·4/0·8, or 1·75. It seems that the heat-flow anomaly has at least two-thirds of its final value. From Table 1 one finds that t_h exceeds 200 m.y.

Table 1. GROWTH OF THE ANOMALY IN TERMS OF θ/θ_∞

t(m.y.)	25	50	100	150	200	250	375	500	750
θ/θ_∞	0·035	0·176	0·418	0·559	0·646	0·705	0·792	0·841	0·893

This conclusion, of course, rests on the assumption that the heat-flow and induction anomalies both arise from the structure of Fig. 2. If their source is a shallower isothermal surface, or if friction at faults contributes significantly to the heat flow, the required age is reduced.

Gilluly's survey[11] of tectonic movements shows uplift in this part of the Rockies from about 300 m.y. ago, when the Ancestral Rocky Mountains were formed by virtually pure uplift, with effectively no compression or vulcanism, to about 220 m.y. ago; and again in Tertiary time between 70 and 20 m.y. ago, with the present Southern Rockies perhaps 15 m.y. old (personal communication from J. Gilluly). An underlying thermal structure 200 m.y. old may very reasonably be connected with these episodes of uplift.

The tectonics of the region abound in unanswered questions. Has North America overrun the East Pacific Rise, or has the latter started under the continent? How are mantle motions associated with spreading from the Atlantic and Pacific combined? We are concerned to show that, where induction and heat-flow anomalies have a common source in the upper mantle, such a source must have considerable age, and this places a boundary condition on the motions. Such a condition seems to apply to the Southern Rockies.

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New Canadian Cryptoexplosion Crater at Lake St Martin, Manitoba

LAKE ST MARTIN (51° 43′ N, 98° 30′ W) forms a broad shallow depression on nearly flat lying Palaeozoic carbonate rocks between Lake Winnipeg and Lake Manitoba. The occurrence of crystalline Pre-Cambrian rocks, fresh looking vesicular volcanic rocks and extensive post-Palaeozoic gypsum deposits within an area of about 200 square miles on the north side of the lake suggests that unusual structural complexities are present. The relations between these rocks are obscured by poor outcrop and extensive swamp, but a series of shallow bore holes in the anomalous area, together with re-examination of the surface outcrop, have recently made it apparent that they are the surface expression of a resurgent cryptoexplosion crater of Permian or Triassic age.

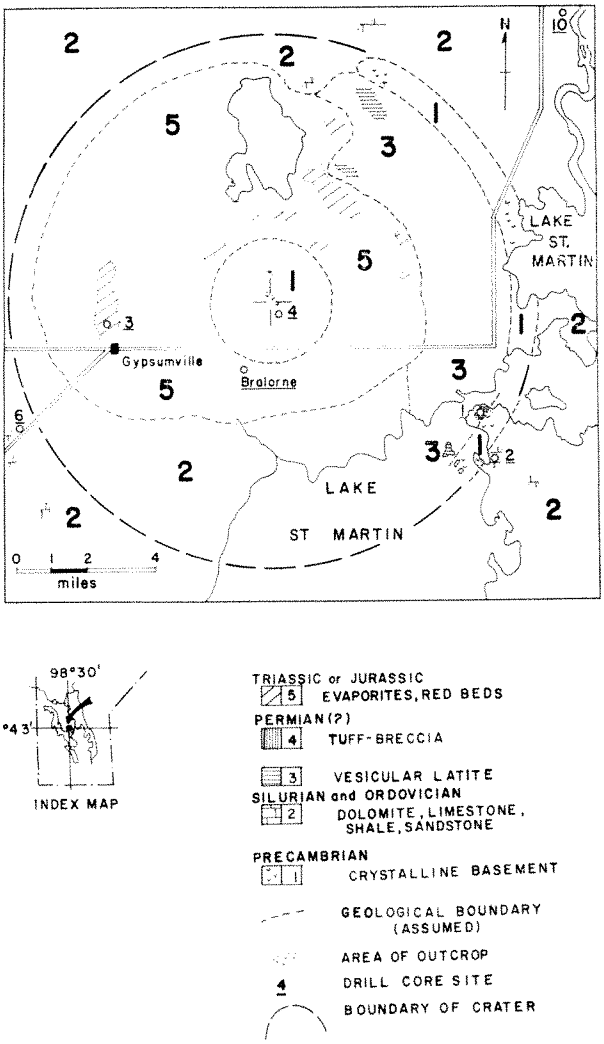

Fig. 1. Geology of the Lake St Martin region.

The geological data for this region are summarized and interpreted in Figs. 1 and 2. Although the data are quite inadequate to fix the structural details, they suggest a central core of crystalline Pre-Cambrian rocks, surrounded by a shallow annular basin of gypsum resting on fragmental rocks of Permian age, which grade outward into near massive igneous latites. In the outer parts of the structure, these rest on disturbed Palaeozoic carbonate rocks, but no Palaeozoic section seems to be present in the central part of the structure. An uplifted "rim" is present around the igneous rocks, locally exposing Pre-Cambrian rocks.

The central Pre-Cambrian core was sampled both in surface outcrop and in bore hole LSM-4 which penetrated 395 feet of similar rocks. The rocks are principally hornblende augen gneiss and granite gneiss, with dikes of spectacular graphic pegmatites. Despite contortion the rocks are little brecciated, and gneissosity is preserved. Pseudotachylite and breccia veinlets up to 4 inches wide occur locally.

Shock metamorphic phenomena are present in all specimens from this core. Quartz is intensely cleaved, and in thin section shows abundant deformation lamellae.

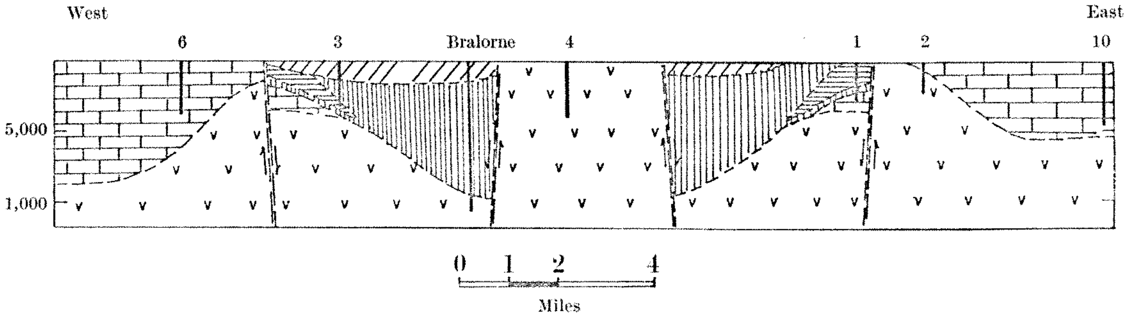

Fig. 2. E–W section across the region of Fig. 1. Vertical exaggeration 14 : 1.

mainly basal but with subordinate omega and prismatic lamellae. As many as five sets of lamellae occur on a single grain. Plagioclase is composed of amoeboid patches interspersed with very fine grained material. Composition, determined by refractive index of fused fragments, varies from An 28 to 40. The optic angle is anomalously small, ranging from 45° to 70°, and refractive indices and birefringence are anomalously small on many grains. Many grains show extreme undulatory or patchy extinction and sinuous patches of maskelynite ($n = 1{\cdot}524$–$1{\cdot}530$). Albite twinning is very rare and, where present, one set of twins is almost invariably isotropic. Amphibole varies from the original green hornblende to a reddish brown material with anomalous brownish interference colour. Birefringence varies from 0·027 to 0·004, and extinction angles from 0° to 12°.

The most contorted rocks consist of very fine grained yellowish schliers, suggesting threads of devitrified glass. Small amounts of clear glass with refractive index near 1·485 (lechatelierite ?) are present in some specimens. Many of the other specimens have an irregular brownish tinge due to partial alternation to analcite and thompsonite.

The Bralorne well, collared 1·8 miles from LSM-4, returned short sections of 4 inch diameter core from depths of 400–1,055 feet. Grey breccia containing fragments of granitoid rocks and very rare fragments of aphanitic purple, igneous rock in a gritty tuffaceous matrix is at least 800 feet thick. It seems to rest on argillized but unshocked granodiorite intruded by stringers of aphanitic igneous rocks. No section of evaporites or Palaeozoic carbonate rocks was penetrated. Fragments in the breccia from the Bralorne well do not display shock phenomena; many are remarkably fresh, suggesting that they are not derived from the altered granodiorite immediately below.

Bore hole LSM-3, collared 4·2 miles west of LSM-4, penetrated 189 feet of sedimentary rocks with gypsum at the top, passing downward into shale and sedimentary breccia which in turn lies with sharp contact on altered polymict breccia containing glassy fragments in a fine clastic matrix (suevite). 96 feet of this breccia is present, grading in the lower 20 feet into an aphanitic purple latite of igneous appearance. This igneous rock with rare small inclusions extends from 265 feet below surface to the bottom of the hole at 299 feet.

Bore hole LSM-1, situated 5·9 miles south-east of LSM-4, passed through 213 feet of remarkably homogeneous, dense, purple, aphanitic latite. Inclusions became common toward the base, and at the base 10 feet of brecciated, rotten, intensely altered, plastically contorted granite are present. The bottom 62 feet of this core consist of sedimentary breccias interbedded with red shale and grey carbonates. Basement fragments are common in the breccias, occasionally with contorted shapes and slight shock metamorphism, but the principal fragments are contorted, discoloured carbonates. The disturbed sedimentary rocks slightly resemble those of the surrounding Ordovician–Silurian carbonate section, but are severely brecciated and altered. They may be part of a Palaeozoic section not preserved elsewhere in this region.

Outcrops of igneous rocks similar to those in LSM-1 occur on an island in Lake St Martin and east of Gypsum Lake. Abundant large vesicles are commonly oriented and indicative of flow. In most observed cases the indicated flow direction was nearly horizontal, but several regions of steep dips, suggestive of fissure vents, were observed. Age determination by the whole rock K/Ar method yields ages of 225 ± 50 m.y. for these rocks[1].

Fresh, unshocked, near-massive granodioritic rocks outcrop intermittently along an arc from east of Gypsum Lake to the east shore of Lake St Martin. Associated with these outcrops of basement rocks are areas of abnormally uplifted Palaeozoic sedimentary rocks. At the site of drill-hole 2 (6·9 miles south-east of LSM-4), the carbonates are uplifted 600–700 feet above their normal position. Drill-hole 6 (7·5 miles south-east of LSM-4) possibly passes through structurally high carbonates as well, but at points as remote from the structure as drill-hole 10 (12 miles north-east of LSM-4), the sedimentary section is normal.

If the curved structural high represented by the Pre-Cambrian outcrops is assumed to be part of the rim of a circular structure, geometric projection from the three main areas of fresh crystalline Pre-Cambrian rocks defines the centre and radius of the area. The results are shown in Fig. 1, and give a diameter of almost exactly 12 miles. The centre falls almost on the site of LSM-4. The diameter of the central mass of shocked Pre-Cambrian rocks cannot be greater than 2·5 miles, and may be less.

The Lake St Martin structure seems to be a typical Canadian cryptoexplosion structure of the resurgent type[2]. Two novel features, however, are displayed: the crater occurs within flat-lying Palaeozoic sedimentary rocks, and a post-crater sedimentary fill is preserved. Canadian craters tend to fall along linear belts uplifted in late Palaeozoic or Mesozoic time[2]. The Lake St Martin structure lies almost precisely on this space–time line but, for reasons unknown, erosion has not yet stripped away the surrounding Palaeozoic rocks as it has at other Canadian craters. The preservation of the gypsum may also be attributed to slight erosion. Gypsiferous crater fills are known in the simple craters Brent and Deep Bay[2], but hitherto no resurgent crater had been discovered which was so pristine that the crater fill was preserved. Erosion of the Lake St Martin structure has apparently been slight. Further deep drilling at the site should produce significant new information on the superstructure of resurgent craters.

Lake St Martin is the third crater the location of which along "welts" on the Pre-Cambrian shield was predicted before discovery (the others being Charlevois[3] and Mistastin Lake[4]). The probability that three structurally and petrographically unusual craters would appear at the predicted sites in the course of less than 100 m.y. as the result of random impact of cosmic missiles seems negligible.

I thank H. R. McCabe and B. B. Bannatyne, Manitoba Department of Mines and Natural Resources, who

arranged access to the outcrops and drill cores, and provided maps and information on the area.

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Composition of some Aluminium Silicate Bearing Contact Rocks from County Donegal, Eire

THE wide occurrence of the three aluminium silicate minerals andalusite, kyanite and sillimanite either individually or in pairs, or sometimes all together in metamorphic rocks[1,2], makes the study of these minerals attractive to petrologists and experimentalists because their presence and associations may be used to delineate the temperature and pressure range of a given metamorphism.

The aureoles associated with the granite plutons of Donegal[3] present a unique opportunity for a study of these polymorphs in the field, particularly because the Donegal area has been investigated in great detail by many workers[4]. The aureoles contain all the polymorphs in various combinations and previous workers in this area have regarded the presence of a particular aluminium silicate or association of the polymorphs to indicate certain limits to the pressure operating during metamorphism. For example, the presence of kyanite in the deformed aureole of the Main Donegal Granite has been taken[3] to indicate that high pressure prevailed. Some undeformed aureoles, which carry andalusite, were thought in contrast to have formed at lower pressures. The role of rock composition was not seriously considered, although Pitcher and Read did suggest that andalusite may be confined to pelites of a particular composition.

Analyses of eighty aluminium silicate bearing pelitic rocks from five of the Donegal aureoles[5] show a remarkable distribution with regard to the aluminium silicates present (Fig. 1). Kyanite-containing rocks have a restricted bulk composition. This mineral is limited to those rocks with magnesium/magnesium + iron ratios greater than about 0·5, while rocks containing andalusite, fibrolite and sillimanite in all possible combinations have unrestricted compositions within the compositional range studied.

Further, new textural and spatial data on the minerals from different aureoles clearly show that the crystallization sequence is kyanite, andalusite, fibrolite, coarse sillimanite[5]. These data are interpreted in terms of a series of overstepping reactions which take place with increasing temperature. The reactions are continuous and no reaction occurs between earlier and later aluminium silicates, all of which develop directly from the groundmass. The presence of more than one polymorph in the same rock is therefore the result of metastable persistence. Not all the rocks show the whole sequence and individual aureoles or parts of an aureole may show only the assemblages formed by one of the reactions.

The first reaction produces kyanite in the relatively magnesium-rich rocks of the Ardara and the Main Donegal Granite aureoles and staurolite in the iron-rich rocks; these latter do not contain kyanite. Later reactions in all the rocks can produce andalusite, fibrolite and sillimanite; they do not involve the formation of an iron-rich phase such as staurolite and occur in rocks showing the whole range of magnesium–iron values found. The actual assemblages seen in a given aureole are complicated by the overstepping which may be marked or limited, depending on the heating characteristics of that aureole. The first reaction is particularly prone, as one might expect, to considerable overstepping so that in the extreme case the assemblages of the first reaction have been found in only one or two rock samples of a particular aureole.

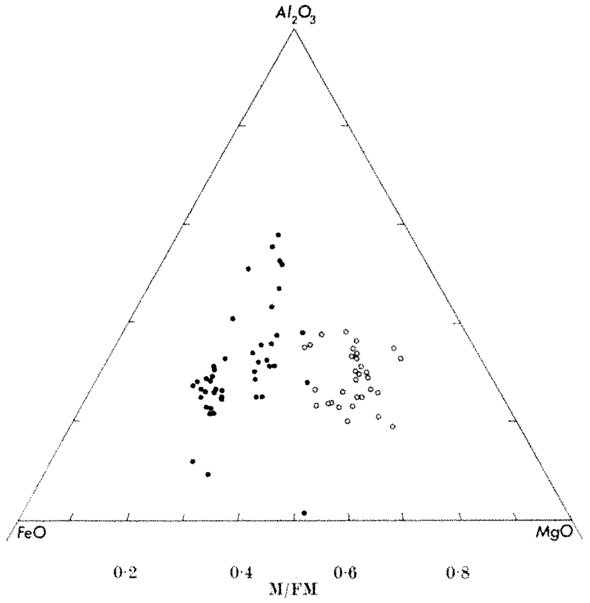

Fig. 1. Thompson AFM diagram of aluminium silicate bearing aureole rocks from County Donegal, Eire. ●, Kyanite free rocks; ○, kyanite bearing rocks.

It may be concluded that the absence of kyanite in some or part of one of the Donegal aureoles may be caused by (1) the lack of an appropriate rock composition—indeed, few pelites have the high M/FM ratio required; (2) the fact that the reaction producing kyanite was overstepped.

Either or both of these factors will result in rocks not containing kyanite, in spite of the fact that the rock may have crystallized in or passed through the stability field of kyanite. In contrast, the absence of sillimanite or fibrolite or andalusite in the Donegal aureoles usually results from the lack of the appropriate physical conditions.

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Experimental Conversion of Biotite to Hydrobiotite

THE weathering of biotite in the soil frequently leads to replacement of interlayer K^+ by hydrated Mg^{2+}, with accompanying structural expansion along the *c* crystallographic axis from 10 to 14 Å (ref. 1). Usually, the 14 Å vermiculite phase becomes established without the development of well-defined intermediate spacings on the X-ray diffraction pattern. Biotite, however, sometimes weathers to hydrobiotite[2] in which there is a regular alternation of 10 Å biotite and 14 Å vermiculite units yielding distinct X-ray reflexions at 12 and 24 Å. Hydrobiotite may also originate, perhaps more frequently[3], through hydrothermal alteration. The vermiculitization of biotite in the laboratory has been extensively studied without observation of an intermediate regularly layered phase[4–6], although in one study[7] hydrobiotite was detected as a minor component. Complete regular interstratification has only been achieved by reversing the reaction so that vermiculite is converted to a mica-like phase by controlled sorption of K^+ and other ions[8–10]. We have now accomplished the transformation of biotite to hydrobiotite in conditions which throw some light on the mechanism involved.

Following a study of biotite weathering at Rehiran, Inverness-shire[11], which suggested that oxidation might play an important part in the transformation of biotite to hydrobiotite, vermiculitization of fresh Rehiran biotite with and without an active oxidizing agent was compared. Two 1 mg samples of biotite flakes (0·5 mm diameter) were immersed in 25 ml. 0·05 M $MgCl_2$ and two drops of bromine (35 mg) were added to one sample only. Both samples were shaken gently for 3 days and then individual flakes examined by X-ray diffraction. With $MgCl_2$ alone, distinct 14 and 10 Å reflexions were visible with no intermediate spacings. The oxidized sample, however, gave a strong line at approximately 12 Å and, for some flakes, a weaker reflexion at 24 Å, indicating hydrobiotite formation. Similar results were obtained using 5 mM $MgCl_2$, but with a 0·5 M solution strong reflexions at 12 Å and 14 Å were found for oxidized samples.

These results indicate that the Rehiran biotite alters to hydrobiotite only when cation exchange proceeds in conjunction with active oxidation. Bassett[12] suggested that the formation of hydrobiotites would be explained if replacement of K^+ by hydrated Mg^{2+} led to strengthened K–O bonds in adjacent interlayers, but he was unable to suggest a mechanism for such a long range effect. A plausible mechanism is suggested by recent observations that K^+ is more firmly held by biotites which have been oxidized either thermally[13] or chemically[14]. Oxidation of the ferrous ions in the two silicate layers on either side of a hydrated interlayer should lead to stronger retention of K^+ in the two adjacent interlayers. Replacement of K^+ by the much larger hydrated Mg^{2+} complex requires considerable flexion of at least one of the adjacent silicate layers, so that random replacement of K^+ in interlayers within the body of a biotite flake will be inhibited by the inflexibility of the surrounding tightly bonded biotite layers. Thus formation of the regular hydrobiotite structure is likely to begin by replacement of K^+ round the edge of a surface layer of the biotite, and to proceed by attack on successive alternate interlayers.

The importance of ferrous iron oxidation in the origin of hydrobiotite is also indicated by the fact that hydrobiotites are frequently, if not always, highly oxidized[15,16]. Moreover, hydrophlogopite, a regularly interstratified Mg rich counterpart of hydrobiotite, has never been recorded as far as we are aware.

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Proton Migration in Hydrogen-bonded Chains

Proton conduction in hydrogen-bonded substances has attracted attention because the observed mobilities are of a comparable order with the electronic mobilities in some semiconductors. Indeed, these materials have been termed protonic semiconductors[1]. The work in this area is motivated mainly by the interest in the proton in biological processes[1]. As the simplest structure in this group, ice with the H_3O^+ defect has been studied intensively[1–5].

Our purpose here is to suggest a possible analogy between the motion of protons in a hydrogen-bonded chain and the motion of dislocations in crystals[6]. This analogy focuses attention on the cooperative motion of many protons and raises the possibility that the actual energy barrier to this cooperative motion is far smaller than that faced by a single proton. In this preliminary examination of the problem, the model used for the hydrogen-bonded chain is kept as simple as possible in order to facilitate direct contact with available computations for the Frenkel–Kontorova dislocation model[7–9].

The model for the hydrogen-bonded chain consists of an infinite one dimensional chain of alternating atomic compounds *A* and ionized hydrogen atoms, similar to the

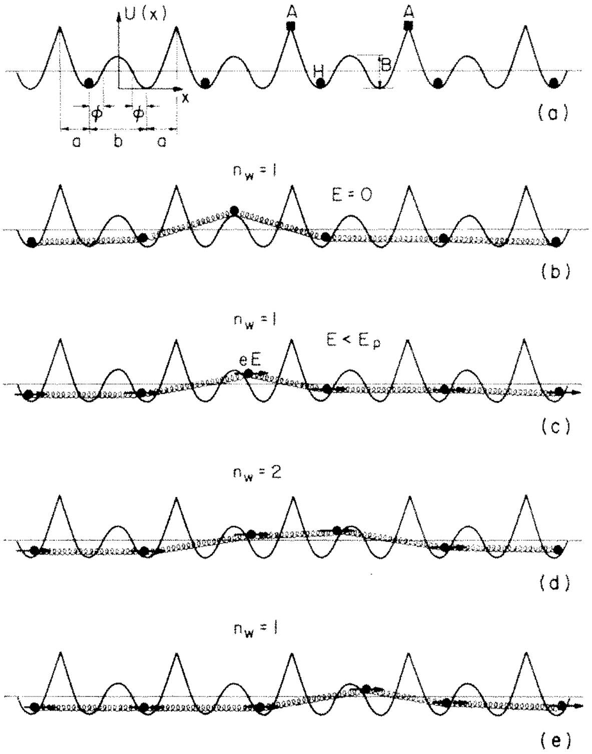

Fig. 1. Model for hydrogen-bonded chain A—H — — A—H — — ; (*a*) Defect-free chain. Horizontal line separates positive and negative curvature regions for $U(x)$. (*b*) Chain with inter-bond defect with no external field. (*c*) Same as (*b*) but with applied field $E < E_p$. Stable configuration. (*d*) Unstable configuration corresponding to conditions of (*c*). (*e*) New stable configuration with defect advanced one step.

one proposed by Fischer, Hofacker and Sabin[2]. This model may represent ice[2] (HO)-H, potassium phosphate[10] ($KHPO_4$)-H, isocytosine[11] ($C_4H_4N_3O$)-H, and so on. The asymmetric position of the protons in the chain of the form

——A–H——A–H——A–H——A–H——A–H——

or alternatively

——H–A——H–A——H–A——H–A——H–A——

is attributed to the existence of a double well potential for the protons; this potential is calculated for ice in ref. 2.

We consider the energy of interaction of the entire collection of A compounds, treated as unstructured points at positions $X_1, \ldots, X_N$, and protons at positions $x_1, \ldots, x_N$. By the Born–Oppenheimer approximation the energy of interaction V may be expressed as a function $V(X_1, \ldots, X_N; x_1, \ldots, x_N)$ which admits the two stable equilibrium configurations indicated above. We regard the positions of the A compounds, $X_1, \ldots, X_N$, as fixed at the equilibrium spacing. Then we postulate that we can write $V = V(x_1 \ldots, x_N)$ in the following form:

$$V = \sum_i U(x_i) + \frac{1}{2} \sum_{i,j} W(x_i, x_j)$$

That is, with the As fixed and uniformly spaced, the interaction energy is regarded as separated into two parts: (1) $U(x_i)$ which describes the interaction of the ith proton with all the As; (2) $W(x_i, x_j)$ which describes the pairwise interaction between the protons.

In order to make direct contact with the dislocation calculations of ref. 7, we assume the following form for $U(x)$, where each x_i is measured from the mid-point between two A locations (Fig. 1a):

$$\begin{aligned} U(x) &= \frac{1}{2} k_2\left(x - \frac{b}{2}\right)^2 & \frac{b}{2} - \varphi \leq |x| \leq \frac{b}{2} + a \\ &= \frac{1}{4} k_2 \varphi b - \frac{k_2 \varphi}{b - 2\varphi} x^2 & |x| \leq \frac{b}{2} - \varphi \end{aligned}$$

For the same reason the proton–proton interaction $W(x_i, x_j)$ is taken as nearest neighbour, corresponding to linear springs with spring constant k_1. By our method of model construction, these springs exert zero force when all the protons are equally spaced in corresponding wells as in Fig. 1a.

In order to introduce the proton defect, we assume that all the protons to the left of a point in the chain have moved into the right wells, but all the remaining protons are still in the left wells. Because of the proton–proton interaction $W(x_i, x_j)$, the equilibrium configuration of the protons will no longer lie at the bottom of the wells of $U(x)$ (Fig. 1b). The problem is now mathematically identical with the dislocation calculations of ref. 7. As shown there, in precisely two equilibrium configurations in this circumstance there will be either n_w or $n_w + 1$ ($n_w = 0, 1, \ldots$) protons in the central portion of $U(x)$ where its curvature is negative. The value of n_w depends on the parameters of the model. The equilibrium solution corresponding to n_w is stable; that corresponding to $n_w + 1$ is unstable. Put another way, the stable solution corresponds to the potential well and the unstable solution to the saddle point in a configuration space with dimension equal to the number of protons.

If an electric field E is applied, exerting a force eE on the protons, there will be again two equilibrium solutions (as shown in the corresponding dislocation calculation[7]), stable and unstable, corresponding to n_w and $n_w + 1$ respectively, provided $E < E_p$ where E_p is discussed subsequently.

The motion of the defect, according to this model, is shown in Fig. 1. In Fig. 1b the stable proton configuration is illustrated, with $n_w = 1$, under an applied field $E < E_p$. The corresponding unstable configuration, with $n_w = 2$, under the same applied field is shown in Fig. 1d. Fig. 1e then shows the defect advanced by one step to the next stable configuration with, again, $n_w = 1$.

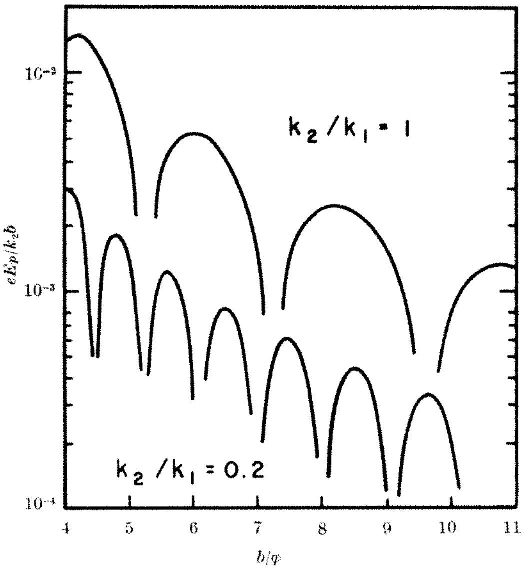

Fig. 2. Critical field E_p as function of b/φ (see Fig. 1) for $k_2/k_1 = 0{\cdot}2$, 1.

The critical field E_p corresponds to the Peierls stress in the dislocation literature[6] and it, along with all other quantities, may be computed explicitly for this model[7–9]. The results for E_p are shown in Fig. 2 for two sets of values of the model parameters.

As shown in ref. 7, the barrier height for the cooperative motion of the protons in this model under an applied field $E < E_p$ is

$$V(E) = B \frac{b}{\varphi} \frac{eE_p}{k_2 b} \left(1 - \frac{E}{E_p}\right)^2$$

where B is the barrier height of $U(x)$.

Fig. 2 shows that the activation energy for cooperative proton motion is several orders of magnitude smaller than B. In fact, for some values of b/φ, $E_p = 0$, and for these critical values there is no barrier to cooperative proton motion. If the protons are now regarded as in thermal equilibrium, the unidirectional transition rate is found, on the basis of a classical analysis[2], to be

$$\mathrm{f}(E) = \frac{(k_2/m)^{1/2}}{2\Pi} \nu e^{-V(E)/kT}$$

where $\nu = \nu(b/\varphi, k_2/k_1)$ is a coefficient of the order of unity. For weak fields, $E/E_p \ll 1$ and, considering the net flux in one direction, the mobility is found as

$$\mu = (\mathrm{f}_{\rightarrow} - \mathrm{f}_{\leftarrow}) \frac{b}{E} = \mathrm{f}(0) \frac{eb^2}{kT}$$

To estimate the transition rate and the mobility of protons in ice, the calculations and graphs given in ref. 2 are taken as the basis of the numerical values. From Fig. 3 of ref. 2, along with the assumption of Coulomb interaction between the next neighbour protons, we estimate

$k_1 = 2{\cdot}94 \times 10^4$ dyne/cm, $k_2 = 2{\cdot}60 \times 10^4$ dyne/cm, $\varphi = 0{\cdot}18$ Å
$b = 0{\cdot}78$ Å, $b + 2a = 2{\cdot}76$ Å, $B = 0{\cdot}0080$ a.u. $= 3{\cdot}47 \times 10^{-13}$ erg.

With the parameters $k_2/k_1 \approx 1$, $b/\varphi = 4{\cdot}3$, we find that $n_w = 1$ from Fig. 2. Then one has $eE_p/k_2b \approx 10^{-2}$ and computes $V(0) = 0{\cdot}0003$ a.u. $= 0{\cdot}13 \times 10^{-13}$ erg. In this case the potential barrier for cooperative motion is about thirty times smaller than the barrier B for individual proton motion. It should be noted that the calculated value of $V(0) \sim 0\cdot2$ kcalories/mole for the cooperative motion is much smaller than the activation energy for conductivity ($\sim 8\cdot5$ kcalories/mole). Thus the major portion of the latter is to be ascribed to dissociation energy, as noted previously[3,4,12]. Table 1 gives the results of the present analysis along with the available experimental data, and in parentheses, the values obtained using B instead of V. In calculating the conductivity $\sigma = qn\mu$, the value of n, the number of proton defects per cm^3, is taken as 10^{10} following ref. 5.

As we indicated at the outset, our purpose was to make direct contact with analogous dislocation calculations and the model has been drastically idealized for this purpose. In particular, the one dimensional representation does not include a mechanism, for example, by suitable defect motion, to reorient the bonds back to the original state to permit d.c. conductivity[13]. Furthermore, the strength of the H–H interaction has undoubtedly been overestimated. On the other hand, the restriction to nearest-neighbours interaction introduces an error which may be compensating. The results seem sufficiently encouraging to warrant further refinement of the model and also its analysis on the basis of quantum rate theory. Here the recent results of Weiner[14] and Weiner and Partom[15,16] may prove useful.

Table 1. RESULTS OF THE ANALYSIS

T (K)	f(s^{-1})	μ(cm^2 V^{-1} s^{-1})	σ(Ω^{-1} cm^{-1})	$\mu_{\rm exp}$	$\sigma_{\rm exp}$
271	$0\cdot98 \times 10^{13}$	$0\cdot11$	$0\cdot18 \times 10^{-9}$	0·8[5]	0·1[3] 3·0[4] 1·3[5] } $\times 10^{-9}$
	($4\cdot3 \times 10^{9}$)	($2\cdot1 \times 10^{-5}$)			

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Preferential Hg^{2}H Formation in the Quenching of Hg(6^3P_1) by H^{2}H

A. B. C. and Hedges[1] have described a technique of monochromatic flash spectroscopy in which an intense pulse of Hg 2537 Å radiation was generated with a microwave discharge. The light was absorbed in a reaction system containing Hg vapour with various quenching gases and transient species were produced at a sufficiently high concentration for their detection by kinetic absorption spectroscopy. We report here the discovery that monochromatic excitation can also be achieved with a specially designed, electroded flashlamp. The method is simple and highly practicable. We have achieved quantal absorptions two-fold greater than that of A. B. C. and Hedges, and the new technique can also be applied with very low pressures of reactants. The determination of the relative yields of mercury hydrides in the quenching of Hg(6^3P_1) by H_2, $^2H_2$ and H^{2}H is described to show application of the method; quenching by H^{2}H was found to produce Hg^{2}H preferentially and this observation indicates a possible mechanism for the reactions.

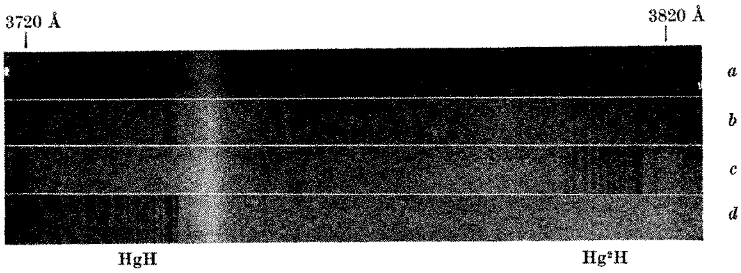

Fig. 1. Mercury hydrides in the reaction of Hg(6^3P_1) with (*a*) H^{2}H, (*b*) $H_2 + {}^2H_2$ (equimolar), (*c*) $^2H_2$, and (*d*) H_2. The (1,0) bands of the $A^2\pi$—$X^2\Sigma^+$ systems of HgH and Hg^{2}H. Each exposure at 16 μs and 300 J flash energy, $[H_2]+[^2H_2] = 2$ torr $+ [Ar] = 700$ torr.

If a flash discharge is made to occur simultaneously between many closely spaced electrode-pairs, we have found that the emitted light consists almost entirely of discrete lines with flash energies up to at least 500 J; the resonance transitions of atoms are intense. Apparently the electron concentration is low compared with that in a conventional flashlamp with a single pair of electrodes; the discharge is silent and of low overall luminosity. With an energy of 300 J per pulse, a plate exposed to fifty flashes showed no detectable background continuum. Optimization of most of the variables, for maximal absorption of the 2537 Å resonance line in a reaction volume, was achieved by constructing and testing various short flashlamps with four to eight electrode pairs. Thus the design of the apparatus, with which the absorption spectra of Fig. 1 were obtained, was realized.

The main body of the device was constructed from a 'Pyrex' tube, length 88 cm and diameter 5 cm. A quartz tube, diameter 1·5 cm, was positioned coaxially inside and was fixed to the 'Pyrex' with glass end-rings and an epoxy resin; the flash discharge was confined to the annulus between the tubes. Forty-four tungsten electrodes were sealed into opposite sides of the 'Pyrex' to form twenty-two opposing electrode pairs. To ensure simultaneous firing, each electrode was isolated with 1 m of coaxial cable[2,3]. The lamp was filled with Hg vapour and 1 torr of helium; it was powered with a 1·5 μF capacitor charged to 20 kV and triggered with a spark gap. The device behaves as an under-damped *LCR* circuit of period 12 μs, exponential half-life of 10 μs, and with a peak current of $\sim 1\cdot5 \times 10^4$ A. A reaction vessel of 5 mm internal diameter was placed coaxially inside the inner quartz tube; optical filters could be located in the intervening volume. The entire apparatus was mounted inside a large furnace and heated to 313 K ($Hg = 6 \times 10^{-3}$ torr) to obtain maximum absorption of resonance radiation. The quantal absorption per pulse was estimated as $1\cdot7 \times 10^{14}$ quanta cm^{-3}, by generating free methyl radicals in the reaction

$$Hg(6^3P_1) + CH_3N_2CH_3 \rightarrow Hg(6^1S_0) + 2CH_3 + N_2$$

The absolute [CH_3] was determined from the integrated absorption of the electronic transition at 2160 Å, for which the *f*-value is known[4].

The spectra of Fig. 1 illustrate the detection of mercury hydrides in the quenching of $Hg(6^3P_1)$ by H_2, $^2H_2$ and H^2H. The (1,0) bands of the $A^2\Pi-X^2\Sigma^+$ system are well separated and the preferential formation of Hg^2H in the quenching of $Hg(6^3P_1)$ by H^2H is clearly indicated. In the equimolecular mixture of $H_2 + {}^2H_2$, the yields of HgH and Hg^2H are approximately equal. Relative quantum yields for mercury hydride formation were measured by plate photometry and by determination of the Franck–Condon factors of the bands. Results are listed in Table 1, where the absolute yield of HgH in the reaction of $Hg(6^3P_1)$ with H_2 is the value recorded by A. B. C. and Hedges[5].

To investigate the initial quantum states of the HgH produced in the reaction of $Hg(6^3P_1)$ with H_2, experiments were conducted down to hydrogen pressures of 0·05 torr. Even at the shortest delay times of ∼7 μs, however, the HgH was detected only with zero-point vibrational energy.

Table 1. QUANTUM YIELDS OF FORMATION OF MERCURY HYDRIDE IN REACTIONS OF $Hg(6^3P_1)$

Reaction	Quantum yields
$Hg(6^3P_1) + H_2 \rightarrow HgH + H$	0·80 ± 0·10
$Hg(6^3P_1) + {}^2H_2 \rightarrow Hg^2H + {}^2H$	0·96 ± 0·10
$Hg(6^3P_1) + H^2H \rightarrow HgH + {}^2H$	0·14 ± 0·02
$Hg(6^3P_1) + {}^2HH \rightarrow Hg^2H + H$	0·80 ± 0·10

The results of Table 1 can be qualitatively understood if it is postulated that $Hg(6^3P_1)$ reacts with H_2 by first inserting between the H atoms to form an electronically excited H HgH*. The excited intermediate subsequently "predissociates" by crossing to a second state of H HgH which correlates with and dissociates to the ground states of HgH + H.

$$Hg^* + H2 \rightarrow H\ HgH^* \text{ (insertion)}$$
$$H\ HgH^* \rightarrow H\ HgH \text{ (crossing)}$$
$$H\ HgH \rightarrow HgH + H \text{ (dissociation)}$$

Predissociation could favour ejection of the lighter H atom in the quenching of $Hg(6^3P_1)$ by H^2H. Such a scheme can therefore account for the non-statistical Hg^2H yield in the H^2H reaction, for the non-statistical total yields of the hydrides and for their apparent production with very little excess vibrational energy.

Extensions of the same experimental method to monochromatic flash spectroscopy with the resonance radiations of cadmium[6], xenon and krypton[7] have been described.

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Constant-Wave Laser Oscillation in a Carbon Monoxide Chemical Laser

CW laser oscillation has been observed on twenty-eight vibration–rotation transitions in the range 5·22–5·55 μm with a CO chemical laser. This is the first report of purely CW lasing in chemically excited CO, although we have previously[1] announced a "quasi-CW" laser of this type in which the duration of the laser pulse is longer than the characteristic decay time of excited CO. We give here the resolved laser spectra and show that long term CW operation can be achieved in a CO chemical laser with rather modest requirements for pump speed and input energy.

The reactions believed responsible for the excitation of CO are

$$O + CS_2 \rightarrow CS + SO + 32 \text{ kilocalories/mole} \quad (1)$$

$$O + CS \rightarrow CO + S + 75 \text{ kilocalories/mole} \quad (2)$$

The excitation mechanism in the CO chemical laser has been controversial[2-4], but Hancock and Smith[5] seem to have confirmed the likelihood of reactions (1) and (2) by studying directly the excitation of CO in these reactions. The fast reaction:

$$S + O_2 \rightarrow SO + O \quad (3)$$

is crucial to the operation of the flash photolysis CO laser and also contributes to generating oxygen atoms. A detailed analysis of the chemistry of this system will be given in a later paper.

The experimental arrangement is shown schematically in Fig. 1. Oxygen atoms were generated by passing a mixture of He and O_2 through a 60 Hz discharge produced with a neon sign transformer rated at 12 kV, 60 mA, controlled by a variable transformer. The atoms were rapidly drawn into the optical cavity where they were allowed to react according to reactions (1) and (2). The reaction products were extracted by a mechanical pump with a rated displacement of 500 l./min. The reaction products were non-corrosive and no special care was necessary except regular changing of pump oil and proper exhaust ventilation. The optical cavity consists of two internal gold-coated mirrors spaced 55 cm apart. One of the mirrors is flat and the other is spherical with radius $R = 230$ cm. The active length is 15 cm long with an internal diameter of 10 mm. Radiation was coupled out through a 0·5 mm hole in the centre of the flat mirror. Output was detected with a cooled (77 K) InSb photovoltaic detector chopped at 500 Hz. The laser spectrum was measured with a 0·5 m Jarrell–Ash spectrometer calibrated in high order with lines from a mercury lamp. Resolution of 0·6 cm^{-1} was obtained with 100 μm slits and a grating blazed at 7·5 μm.

Twenty-eight lines were observed and assigned to *p*-branch transitions in the CO molecule (Table 1). The constants used in computing the theoretical values of the

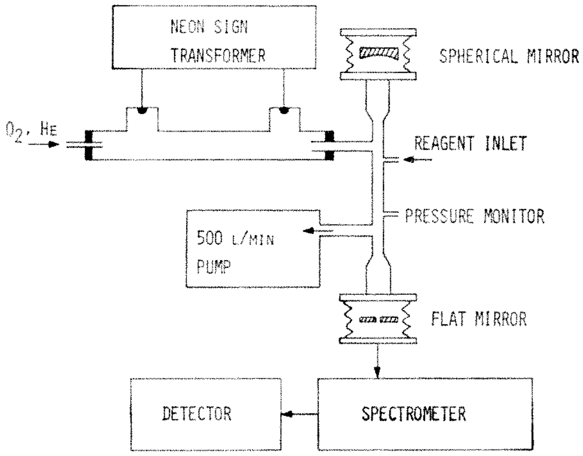

Fig. 1. CW carbon monoxide chemical laser.

Table 1. OBSERVED LASER TRANSITIONS IN CHEMICALLY EXCITED CARBON MONOXIDE

ν Observed (±0·60 cm⁻¹)	Assignment vibration rotation	ν Observed − ν Calculated
1,914·35 cm⁻¹	8– 7 *p*(12)	+0·45 cm⁻¹
1,909·97	8– 7 *p*(13)	+0·12
1,905·66	8– 7 *p*(14)	−0·19
1,901·71	8– 7 *p*(15)	−0·07
1,898·03	8– 7 *p*(16)	+0·36
1,892·67	9– 8 *p*(11)	+0·40
1,888·48	9– 8 *p*(12)	+0·15
1,884·18	9– 8 *p*(13)	−0·17
1,880·32	9– 8 *p*(14)	−0·04
1,876·68	9– 8 *p*(15)	+0·36
1,872·72	9– 8 *p*(16)	+0·48
1,866·76	10– 9 *p*(11)	+0·02
1,862·68	10– 9 *p*(12)	+0·15
1,859·03	10– 9 *p*(13)	+0·14
1,855·35	10– 9 *p*(14)	+0·43
1,851·36	10– 9 *p*(15)	+0·44
1,846·91	10– 9 *p*(16)	+0·03
1,844·97	11–10 *p*(10)	−0·15
1,842·71	10– 9 *p*(17)	−0·11
1,837·66	11–10 *p*(12)	+0·26
1,834·06	11–10 *p*(13)	+0·56
1,830·09	11–10 *p*(14)	+0·52
1,825·69	11–10 *p*(15)	+0·09
1,821·52	11–10 *p*(16)	−0·08
1,812·60	12–11 *p*(12)	+0·54
1,808·44	12–11 *p*(13)	+0·25
1,804·27	12–11 *p*(14)	−0·03
1,800·42	12–11 *p*(15)	+0·06

transitions are taken from ref. 6. Individual lines were quite unstable because the Doppler linewidth and the spacing between adjacent longitudinal modes are comparable for the experimental conditions listed here. Quite often, one mirror had to be tuned so that a cavity resonance would lie under the fluorescent line, making it impractical to scan the spectrometer. Spectra were recorded by advancing the spectrometer in steps of 0·5 cm⁻¹ and tuning the cavity for maximum output at each spectrometer setting. The region 5·0 to 5·6 μm was investigated and it is possible that a few transitions in this range were suppressed because of failure to satisfy the cavity resonance condition.

In optimum conditions, a total CW output of the order of 1 mW was obtained. This required an input of approximately 400 W into the oxygen dissociator. No effort was made to cool the walls of the laser tube although they became quite hot (~80° C) during operation, because of the chemical reaction. Thermal tuning of the optical cavity and mechanical vibrations are responsible for a slight instability in the total output. Although the total output of the laser was rather stable, individual lines were affected strongly by the thermal tuning caused by the cavity resonance conditions mentioned above.

The output power seems to be limited only by the oxygen atom flow rate. The oxygen atom flow rate was determined from a gas phase titration[7] with NO_2 at the reactant inlet and was found to be $\sim 2\times10^{-4}$ moles/s. In optimum lasing conditions, p (He) $\simeq$ 10–15 torr and p (O_2) $\simeq$ 1 torr. The NO_2 flow rate was measured with a calibrated flowmeter.

By using more efficient coupling, more effective oxygen atom production and transverse flow, significant increases in the output power are envisaged.

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Anodic Reactions of the Hydrogen Electrode

EXTENSIVE studies have been made of the cathodic reactions of the hydrogen electrode (see reviews[1,2]) but relatively few observations have been made in anodic conditions[3–5]. One reason why the anodic reactions of the hydrogen electrode received little attention

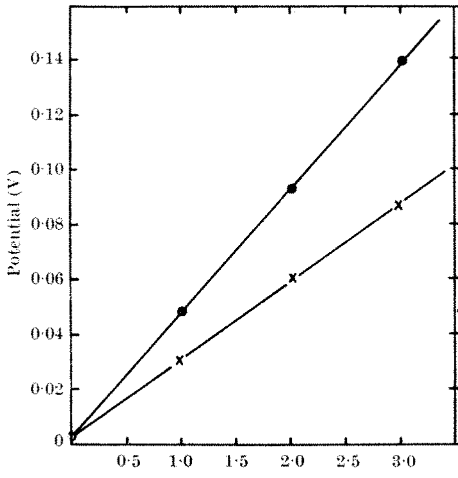

Fig. 1.

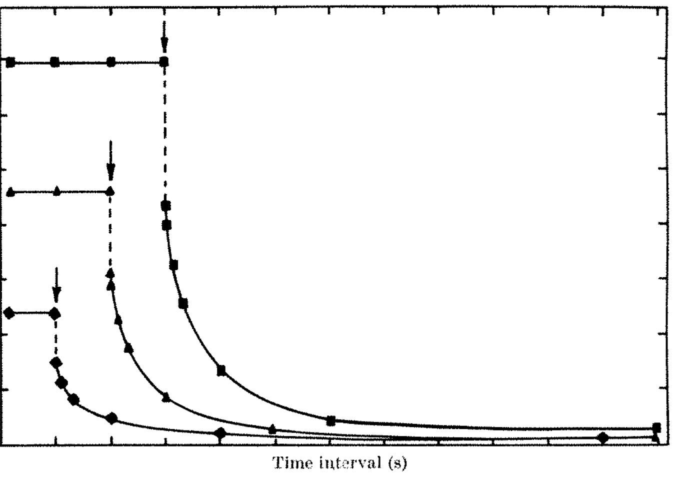

Fig. 2.

Fig. 1. Potential of hydrogen electrode at various current densities at 60° C. ●, Observed potential; ×, observed potential minus resistance polarization.

Fig. 2. Decay of potential on interrupting the current at the hydrogen electrode. Arrows indicate current interrupted; – – – –, instantaneous decay; ◆, 1·00 mA mm⁻²; ▲, 2·00 mA mm⁻²; ■, 3·00 mA mm⁻².

was that hydrogen electrodes which would support substantial anodic currents were not available. More recently, however, the interest in fuel cells has stimulated the development of electrodes that could maintain large currents in these conditions and as a result the anodic properties of the hydrogen electrode have been elucidated.

Studies of the cathodic reactions of the hydrogen electrode invariably concluded that the overpotential was related to the logarithm of the current, and this well-known "Tafel" relationship has become the basis of modern electrochemical theory[6]. In contrast the more recent work with hydrogen fuel cell electrodes[7-10] showed that in anodic conditions there was a linear relationship between the potential and the current. This linear relationship may be a result of the resistance component of the overpotential, which would be directly proportional to the current, being so large as to mask the true relationship for the reaction overpotential. To establish whether this linear relationship is characteristic of the electrode reaction it is thus necessary to determine the relationship between the potential and the current when the resistance component has been eliminated. At the same time it should be appreciated that these characteristics of fuel cell electrodes can only contribute to an understanding of the kinetics of the anodic reactions of hydrogen electrodes if the fuel cell electrodes are truly reversible hydrogen electrodes.

We have attempted to resolve the situation in the case of fuel cell electrodes consisting of palladium (3 per cent) supported on active carbon bound with PTFE[11]. The resistance polarization at 60° C of a circular electrode (3 mm × 57 mm in diameter) was readily measured in potassium hydroxide (8 M) by interrupting the polarizing current at different potentials and observing the decay of the potential on an oscilloscope. The results are summarized in Figs. 1 and 2, which show that the resistance of the electrode system accounted for about 30 per cent of the total polarization; the dotted line in Fig. 1 shows that the resistance free polarization is linearly related to the current density.

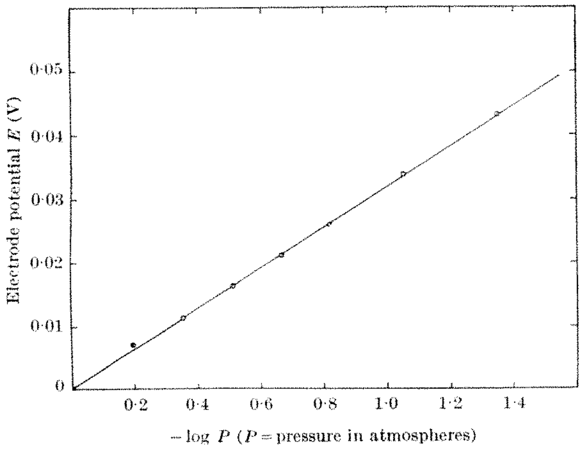

Fig. 3. The effect of hydrogen pressure on a fuel cell electrode at 25° C. ———, Theoretical line $E = -0.030 \log P$; ○, experimental points.

The reversibility of fuel cell electrodes was tested by ascertaining whether the open circuit potential was related to the pressure of hydrogen according to the Nernst equation. This was possible with an apparatus used in other studies of anodic reaction of the hydrogen electrode[12]. The results are shown in Fig. 3, where the open circuit potential is plotted against the logarithm of the hydrogen pressure. It can be seen that the results are in good agreement with the theoretical line.

It can thus be concluded that fuel cell electrodes of the type used in these experiments behave as reversible hydrogen electrodes and that in anodic conditions the potential is linearly related to the current.

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Low-cost Oxygen Electrode Material

There is at present great interest in high energy density batteries, especially for use in urban transport[1]. For this application an electrically rechargeable system would be ideal, and night charging would assist in levelling out the demand for electricity. Metal-air batteries (notably zinc-air) are probably nearest to practical usefulness, but one limitation to their large scale use is the fact that a noble metal catalyst (usually platinum) may need to be used in the air electrodes, which have to reduce oxygen from the air to OH^- ions in the concentrated alkali electrolyte and subsequently to act in reverse for recharging. I report here preliminary tests in which lanthanum cobaltite—a semiconducting oxide—has given results comparable to those produced by platinum. The material clearly deserves detailed further attention and, after discussion of the results, suggestions are made for its development into a practical electrode.

The activity of lanthanum cobaltite ($LaCoO_3$) has been discovered during an investigation of semiconducting oxides as potential oxygen electrode materials. Attention has concentrated particularly on oxides with high conductivity ($> 10^3$ Ω^{-1} m^{-1}) to permit the use of a bulk electrode of an active material. Such a form would be ideal as it should have lower manufacturing costs than a thin-layer hydrophobic construction, and it would minimize any corrosion problems.

The oxides have been examined as 6 mm diameter rotating disk electrodes, chiefly at room temperature, with an automatic potential sweep rate of 12 mV/min. With a planar rotating electrode rather than a more practical arrangement, only small currents are obtained, but there is no possibility that the lack of activity of an electrode may result from too small an effective surface area.

So far NiO, CoO, SnO_2, In_2O_3, $LaCrO_3$, $LaCoO_3$, $PrCoO_3$ and $SrMoO_4$ have been examined, all but $SrMoO_4$ doped with aliovalent impurities. Only the cobaltites have given encouraging results, and $LaCoO_3$ is to be preferred to $PrCoO_3$ because of the much higher cost of praseodymium compounds. The conductivity of $LaCoO_3$ doped with strontium or nickel can be made greater[2] than 10^4 Ω^{-1} m^{-1}, and the material is stable in alkali although it dissolves readily in acid. A typical polarization curve of lanthanum cobaltite doped with 20 mole per cent Sr ($La_{0 \cdot 80}Sr_{0 \cdot 20}CoO_3$) is given in Fig. 1, which compares it with curves for a

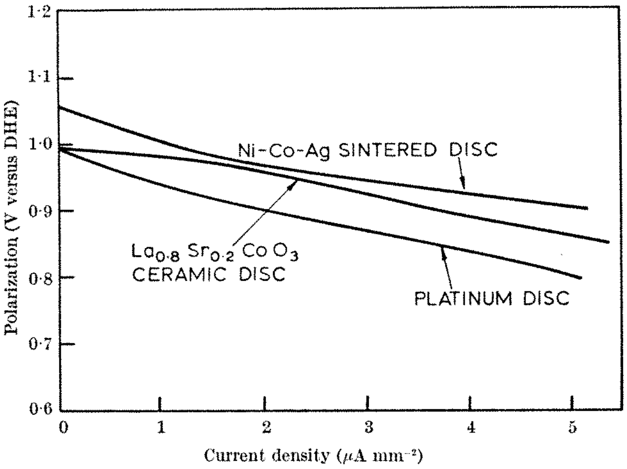

Fig. 1. Oxygen reduction in 5 N KOH solution at 25° C for $La_{0\cdot8}$ $Sr_{0\cdot2}CoO_3$ ceramic, Ni–Co–Ag metal sinter, and platinum sheet (rotating electrode speed 1,500 r.p.m., voltage sweep 12 mV min⁻¹).

platinum disk and for a porous metal sinter of Ni, Co and Ag developed at CERL by Hart and Wrench (private communication). At a given geometric area current density, the polarization of all three systems is similar. The results are not always completely reproducible, possibly because of impurities in the solution, but appreciable activity is always obtained. The metal composition has already been developed with simple manufacturing techniques into a practical porous sinter, from which adequate current densities are obtained.

Both strontium-doped and nickel-doped lanthanum cobaltite give high activity, so the activity is unlikely to be a direct effect of the impurity, especially because the strontium and nickel substitute for the lanthanum and cobalt respectively. Moreover, because $PrCoO_3$ too is very active whereas $LaCrO_3$ is not, even though, like $LaCoO_3$, they have the perovskite structure, the activity seems to be a particular attribute of the cobalt ions. (It is noteworthy that the metal sinter also uses cobalt as an active element.) There are two immediate differences between $LaCrO_3$ and $LaCoO_3$ which may be important in determining that $LaCoO_3$ is active:

(*a*) Whereas $LaCrO_3$ shows only *p*-type conductivity, $LaCoO_3$ can easily be made either *p* or *n*-type and also has a band gap[2] of only 0·30 eV. This amphoteric behaviour and narrow band gap may aid the absorption of oxygen and the subsequent release of ions. The electron mobility in $LaCoO_3$, however, is only one tenth that of the holes[2], and less impurity can be substituted (Gerthsen and Hardtl[3] reported that the solubility of thorium is only 4 per cent), so that the attainable conductivity is much lower. Within these limitations the extension of the measurements into the *n*-type regime will be very valuable.

(*b*) $LaCrO_3$ is antiferromagnetic, with a Néel temperature of 320 K; $LaCoO_3$ shows no long range magnetic order, but its detailed magnetic structure is still under discussion[4]. Winter[5] has attributed an improvement in the chemisorption of oxygen on to nickel oxide at high temperatures to the fact that the material is then above its Néel temperature. $LaCrO_3$ certainly becomes more active above its Néel temperature, but it is not yet clear whether this increase can be correlated with the magnetic transition.

In large quantities the material cost of doped $LaCoO_3$ is about £2 per kg. Further reduction in material costs would be achieved if "technical grade" ceria could be substituted for pure lanthanum oxide, for it contains 23 per cent La_2O_3 as well as 45 per cent CeO_2 and 18 per cent Nd_2O_3, with the balance comprising other rare earths. All rare earth cobaltites have the perovskite structure[6], and technical grade ceria should therefore be effective in making a practical electrode, in which case it would halve the materials' cost. Two main methods of electrode manufacture are being considered: thin porous plates, and a fine particle form for use in hydrophobic electrodes. There are various techniques for producing fine particle sizes[7], such as precipitation from aqueous solutions of the salts, or freeze drying (see, for example, ref. 8), which is becoming a powerful technique for catalysts.

In conclusion, $LaCoO_3$ deserves further detailed attention, both for its technical development into a practical electrode, and also because its electrical properties make it a unique material for investigating the basic processes of oxygen reduction.

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Comments on "The Flow of Non-Newtonian Fluids under a Varying Pressure Gradient"

Barnes, Townsend and Walters[1] have communicated some results concerned with the pulsating flow of a non-Newtonian fluid through a pipe, in which it was shown that the mean pressure gradient along the pipe axis in pulsating flow conditions can be less than the constant gradient in conditions of steady flow, and it is suggested that this may be of industrial importance.

The driving pressures required for transporting fluids considered by the above authors are moderate and introduce no technological difficulties. It is much more important to find out whether there is an economic advantage to be gained with pulsating flow, and this is a different problem.

Thus if the flow rate Q is given by

$$Q = Q_m + Q_1 \cos \omega t \tag{1}$$

and the pressure gradient by

$$P = P_m + \sum_{n=1}^{N} P_n \cos (n\omega t - \varphi n) \tag{2}$$

then the pumping power is equal to

$$P_m Q_m + \frac{P_1 Q_1}{2} \cos \varphi_1 \text{ (unit pipe length)} \tag{3}$$

while the transport energy per unit volume of fluid is equal to

$$P_m + \frac{P_1 Q_1 \cos \varphi_1}{2Q_m} \tag{4}$$

In some cases the second term in equations (3) and (4) may be significant. Furthermore, even when power would be reduced or energy saved by oscillatory pumping it is, of course, worthwhile to adopt this technique only if the additional cost of the oscillatory equipment is not too large, as Fig. 1 indicates. Preliminary investigations have already been made by Harris[2] in which results of the type communicated by Barnes, Townsend and Walters

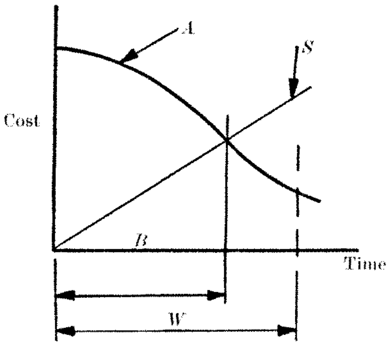

Fig. 1. Economic factors in split-energy pumping. *A*, Addition cost of oscillatory equipment (initial cost + maintenance + depreciation). *S*, Saving in energy cost. *B*, Break-even time. *W*, Oscillatory equipment write-off time.

are foreshadowed. The experimental analytical equipment is also available[3,4].

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BIOLOGICAL SCIENCES

Excretion of Murine Leukaemia Virus

THE study of mice experimentally infected with murine leukaemia virus (MuLV) has revealed leukaemogenic activity in the milk, saliva, urine and faeces, and typical MuLV particles in the mammary glands, salivary glands, and pancreas. MuLV has not been demonstrated, however, in the excretions or secretions of naturally infected mice[1-3]. The development by Hartley *et al.*[4,5] of the complement fixation test for murine leukaemia virus provided a sensitive *in vitro* assay for MuLV and a practical means for the detection of virus in tissues, mouth swabs and excretions. We report here studies on the isolation of virus from excretions and secretions of naturally infected high leukaemia strains AKR/J, C58/J and SJL/J.

The virus isolation studies were conducted on 6 to 10 month old retired breeders from the production colonies of the Jackson Laboratory. They were housed, twenty-five to a cage, in stainless steel cages (12 × 24 × 6·5 inch), provided with white pine shavings and hardwood chips for bedding. The cages were cleaned weekly; food and water were provided *ad libitum*. Mouth swabs were obtained by rolling cotton swabs in the mouth until they became moistened. Urine and faeces were collected on fresh strips of aluminium foil, and blood samples were obtained from cuts in the tail made with razor blades disinfected with alcohol. Specimens were taken at 14-day intervals from four to eleven mice of each strain, approximately half from males and half from females. They were placed in vials containing 1 ml. of Eagle's minimum essential medium with 200 units of penicillin and 200 μg of streptomycin per ml., and stored at −70° C until they could be tested. All specimens were tested individually.

The complement fixation assay for MuLV involves inoculation of secondary mouse embryo cell cultures from NIH Swiss mice and Fischer rat embryo cell cultures as already described[4-6]. After incubation for 21 days the cells were scraped off the plates, suspended in approximately 0·6 ml. of the culture fluids and sonicated gently. The resulting cell packs were assayed for the group specific MuLV antigen by the complement fixation test against anti-Moloney sarcoma virus rat sera.

The results are presented in Table 1: MuLV was detected in four of forty-four AKR/J faecal specimens; six of the forty-four AKR/J blood specimens were positive, indicating that they were viraemic. The positive sample of rat embryo cell culture differed from those on mouse embryo cell culture. We isolated MuLV from three of thirty-six C58/J blood specimens, but did not detect virus in any of twenty-four SJL/J samples, although Hartley consistently isolated virus from SJL/J spleen extracts[5]. Thus naturally infected AKR/J mice do excrete leukaemia virus in the faeces and MuLV can be recovered without further concentration techniques such as ultracentrifugation and ultrafiltration[7].

Table 1. MURINE LEUKAEMIA VIRUS ISOLATED FROM BLOOD, SALIVA AND EXCRETIONS

	Mouth		Urine		Faeces		Blood	
Strain	METC*	RETC†	METC	RETC	METC	RETC	METC	RETC
AKR/J	0/44‡	0/26	0/30	0/20	3/44	1/26	5/44	1/26
C58/J	0/36	0/24	0/35	0/32	0/36	0/24	3/36	0/24
SJL/J	0/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24

* Mouse embryo tissue culture.
† Rat embryo tissue culture.
‡ No. isolations/No. mice sampled.

Our findings seem relevant to the question of the horizontal transmission of MuLV, and are consistent with those of Aoki *et al.*, who observed the development of soluble Gross virus antigen (GSA) in GSA negative C3Hf/Bi mice, and the appearance of GSA antibody in GSA negative C57Bl/6 mice after these were placed in cages with GSA positive AKR mice[8].

According to available evidence, the transmission of MuLV occurs chiefly vertically as a covert viral genome[9]. By contrast, horizontal spread is both infrequent and unimportant (ref. 9 and unpublished observations of R. J. H., W. T. Lane, H. M. and D. D. M.). Most, if not all, inbred mice carry vertically transmitted, that is, inherited, RNA tumour virus information (refs. 9, 10 and unpublished observations of R. J. H., W. T. Lane, H. M. and D. D. M.). The degree of "virus expression" depends largely on host genetic factors[9,11]. Complete, infectious forms of MuLV have been found in only few inbred strains, and in most the virus is covert or repressed to varying degrees (ref. 9 and unpublished observations of H. M., D. D. M. and R. J. H.). AKR/J and C58/J mice are two strains with full expression of virus characterized by the presence in tissues of complete, infectious MuLV. Our experiments demonstrate by direct isolation the presence of complete virus in excretions and secretions. Because the viral envelope on completion at the cell surface acquires host cell-specific components, including major histocompatibility (*H2*) antigens, strains of mice differing in *H2* genotype are resistant to horizontal transmission of excreted MuLV (refs. 12-16 and unpublished observations of H. M., D. D. M. and R. J. H.). We therefore interpret the findings of Aoki *et al.*[8] as evidence for an accelerated rate of indigenous virus expression influenced by the stress of exposing mice of two different strains to one another, rather than as proof for horizontal virus spread. Certain strains of mice develop viral envelope (neutralizing) antibodies spontaneously. Even in the case of Law[2], who reported that the urine and faeces of mice infected with the Moloney leukaemia virus remained leukaemogenic (by *in vivo* assay) after 3 h exposure to room temperature, the actual transmission of virus cannot be distinguished from activation of endogenous MuLV. Thus, the fact that certain "high leukaemia" strains with full expression of viral function excrete "infectious" virus has little significance as a mode of viral spread. Rather, it may represent a defence mechanism of the host for shedding unintegrated cellular contaminants.

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Inhibition of Glycoprotein Synthesis in L5178Y Mouse Leukaemic Cells by L-Asparaginase *in vitro*

L-Asparaginase (EC 3.5.1.1), which hydrolyses the amide group of asparagine, inhibits certain neoplasms[1-4] and is used for treating human acute leukaemias[5,6]. Although asparaginase sequentially inhibits protein, RNA and DNA synthesis[7], its cellular action is undefined. We have investigated the effects of asparaginase on the synthesis of carbohydrate–amino-acid linkages in glycoproteins which involve the amide group of asparagine and N-acetyl-glucosamine residues[8]. Our preparation of L-asparaginase (from *Escherichia coli*, 321–363 U/mg protein) was free of contaminating enzymatic activity, although it was slightly active (2·1 per cent of the activity towards asparagine) with glutamine as substrate. The preparation was free of glycosidase and proteolytic activities[9,10].

L5178Y cells (mouse leukaemia cell line) were grown in exponential phase in sealed containers in Fischer's medium[11]. Protein was determined by the method of Lowry *et al.*[12]. L5178YF cells grown in identical conditions were carried in CDF$_1$ mice before transfer to culture. Fetuin[13] and porcine submaxillary gland glycoprotein[14,15] were prepared and determined as previously described[16].

The data in Fig. 1 show that glycoprotein and protein synthesis in L5178YF cells were inhibited by L-asparaginase. Inhibition of glycoprotein synthesis (66 per cent in 20 min) was more marked than inhibition of protein synthesis (30 per cent in 20 min).

Table 1. TRANSFER OF N-ACETYLGLUCOSAMINE FROM UDP-N-ACETYLGLUCOSAMINE INTO ENDOGENOUS AND EXOGENOUS ACCEPTORS IN L5178Y CELLS AND ASPARAGINASE-TREATED L5178Y CELLS

	C.p.m./mg protein	
	Endogenous	Exogenous
L5178Y	4,344 ± 21	6,139 ± 29
L5178Y + asparaginase	2,591 ± 16	6,241 ± 73

Two groups of 10^6 L5178Y cells in 10 ml. of Fischer's medium were incubated for 1 h at 37° C, one with 1,000 units of L-asparaginase (L5178Y + asparaginase) and one with 0·1 M Tris buffer (*p*H 7·6). After the incubation the cells were centrifuged at 5,000*g* and the pellet homogenized in 0·1 per cent 'Triton X-100' in a Ten Broeck homogenizer. This extract was then used for assay of transfer of N-acetylglucosamine into endogenous and exogenous acceptors. The assay for endogenous activity contained: 100 μl. of the 0·1 per cent 'Triton X-100' extract, 10 μl. of 0·1 M $MnCl_2$, 50 μl. of glass distilled water, and 10 μl. of the UDP-N-acetyl-[^{14}C]glucosamine (25 nCi, approx. 6×10^{-11} mole) to a final volume of 0·170 ml. Incubation was 37° C for 1 h, after which radioactivity was determined by methods previously described[17]. The assay system for exogenous activity contained the above plus 50 μl. of the fetuin acceptor in place of the water. The acceptor for the exogenous assay was made by removing sialic acid, galactose and N-acetylglucosamine from fetuin[18]. Means ± 1 *S.D.*

Asparaginase-treated cells did not synthesize appreciable amounts of protein or glycoprotein after 20 min (Fig. 1). Hydrolysis and chromatography of the radioactive products[17] indicated 100 per cent recovery of the respective leucine and fucose fractions. Similar experiments on L5178Y cells (Fig. 2) indicate that incorporation of aspartic acid into protein was inhibited later, and to a lesser extent than was glycoprotein synthesis.

High levels of asparaginase did not completely inhibit protein or glycoprotein synthesis; a residual, asparaginase-resistant synthesis was observed. Presumably this involved synthesis of proteins devoid of L-asparagine and glycoproteins not containing the asparagine–N-acetylglucosamine linkage. Preincubation with L-asparaginase (Fig. 2) for 1 h before measurement of glycoprotein synthesis gave results no different from those obtained when enzyme was given simultaneously with the labelled precursors. This indicates that the enzyme affected nascent synthesis and not existing glycoprotein precursor pools. In these experiments, leucine and fucose were recovered 100 per cent; glucosamine was recovered 70 per cent as glucosamine, 20 per cent as galactosamine, and 10 per cent as N-acetylneuraminic acid.

The transfer of N-acetylglucosamine to endogenous acceptors was impaired by incubation of L5178Y cells

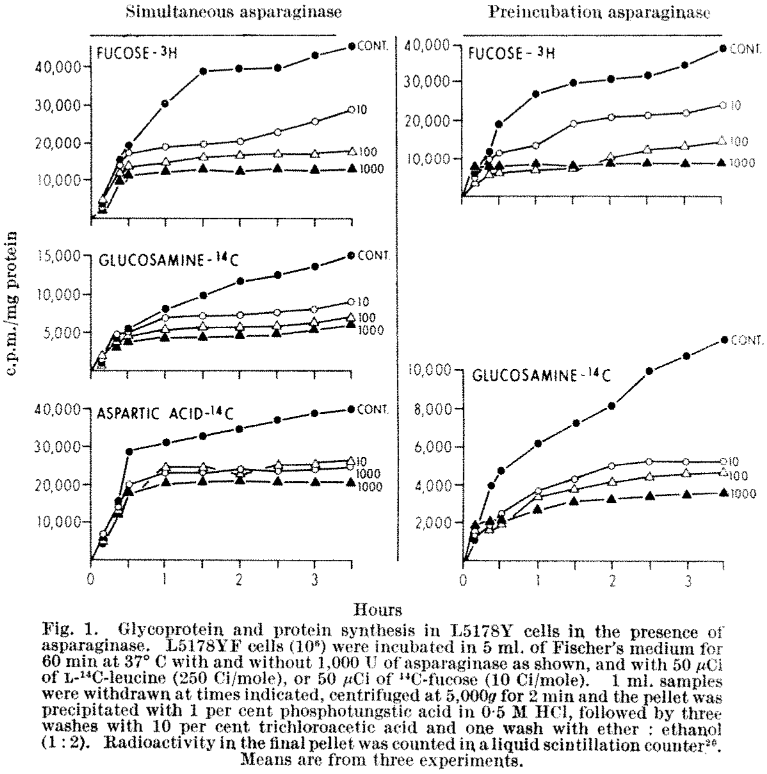

Fig. 1. Glycoprotein and protein synthesis in L5178Y cells in the presence of asparaginase. L5178YF cells (10^6) were incubated in 5 ml. of Fischer's medium for 60 min at 37° C with and without 1,000 U of asparaginase as shown, and with 50 μCi of L-^{14}C-leucine (250 Ci/mole), or 50 μCi of ^{14}C-fucose (10 Ci/mole). 1 ml. samples were withdrawn at times indicated, centrifuged at 5,000*g* for 2 min and the pellet was precipitated with 1 per cent phosphotungstic acid in 0·5 M HCl, followed by three washes with 10 per cent trichloroacetic acid and one wash with ether : ethanol (1 : 2). Radioactivity in the final pellet was counted in a liquid scintillation counter[26]. Means are from three experiments.

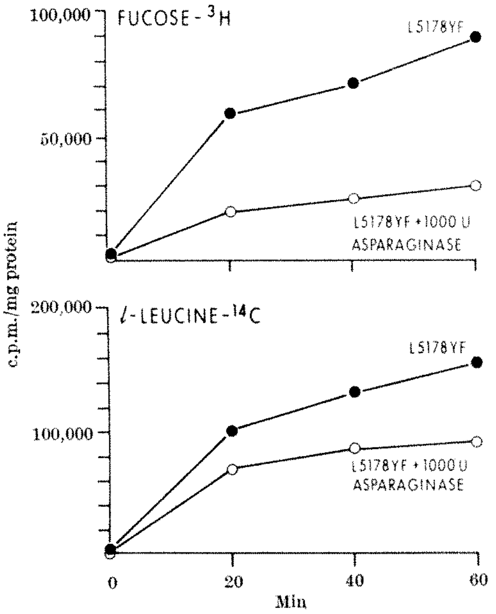

Fig. 2. Glycoprotein and protein synthesis in L5178Y cells in the presence of asparaginase. L5178Y cells (3×10^6) were incubated in 10 ml. of Fischer's medium with the indicated units of asparaginase, and either 50 μCi of ^{3}H-fucose (10 Ci/mole), 10 μCi of ^{14}C-aspartic acid (100 Ci/mole) or 10 μCi of ^{14}C-glucosamine (50 Ci/mole). For experiment 1, isotopically labelled compound and L-asparaginase were added together; for experiment 2, cells and L-asparaginase were incubated for 1 h before addition of the isotopically labelled compound. Protein bound radioactivity was determined as given in Fig. 1. Experiment 1 (top) and experiment 2 (bottom).

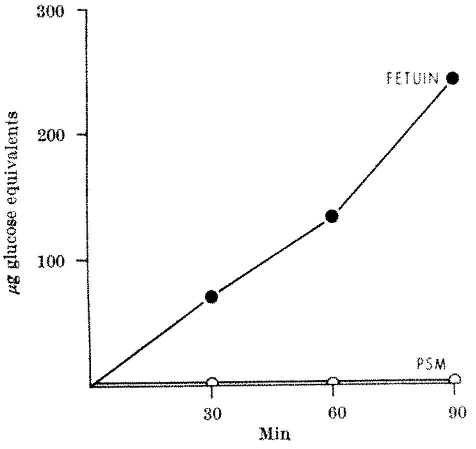

Fig. 3. Release of carbohydrate from fetuin by asparaginase. Fifty mg of fetuin or porcine submaxillary gland glycoprotein (PSM) was incubated at 37° C with 1,000 U of asparaginase in 1 ml. of 0·1 M Tris (pH 7·6). At indicated times 0·25 ml. of the reaction mixture was removed and 0·75 ml. of 10 per cent trichloroacetic acid was added. This mixture was centrifuged at 32,000g for 10 min and the supernatant analysed (anthrone test). Data are given as μg glucose equivalents in 7·5 per cent trichloroacetic acid. Means are from three experiments. Controls, which gave no release of glucose equivalents, included fetuin alone, porcine submaxillary gland glycoprotein incubated alone, asparaginase incubated alone, or either substrate incubated with 1,000 U of L-asparaginase previously incubated at 100° C for 1 h.

with L-asparaginase (Table 1). The inhibition was 40 per cent; thus 60 per cent of the acceptors were available—presumably those not directly requiring the amide nitrogen of asparagine. When exogenous acceptors were added to the reaction, both enzyme-treated and control preparations had identical activity, so that the acceptors and not the transferase enzymes were impaired by L-asparaginase. Hydrolysis of the radioactive product indicated recovery of 100 per cent as unchanged N-acetyl-[^{14}C]glucosamine.

L-Asparaginase released carbohydrate from fetuin but not from porcine submaxillary gland glycoprotein (Fig. 3). This action of L-asparaginase could cause cleavage of formed glycoproteins in cell membranes.

Because residual glutaminase activity is present in the asparaginase preparation, and high levels of asparaginase were used in the experiments, the results could be explained in part by depletion of glutamine residues as opposed to asparagine residues. It is clear, nevertheless, that L-asparaginase has the ability to cleave formed glycoproteins and to inhibit glycoprotein synthesis as well as extensively inhibiting protein synthesis. Because membrane glycoproteins turn over rapidly[19], either action could cause membrane lysis and cell death. The activity of L-asparaginase towards certain tumour cells may be caused, in part, not only by absence of L-asparagine synthetase but also may be related to the increased glycoprotein transferase activity—and therefore glycoprotein turnover—found in cells which have undergone oncogenic transformation[20]. The results are of interest in the light of those recently described[21] in which L-asparaginase was found to suppress the immune system, for immunoglobulins are glycoproteins; moreover, glycoproteins may be necessary for the rapid proliferation of lymphoid cells.

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Synthesis of Herpes Simplex Virus Structural Proteins in Arginine Deprived Cells

Arginine deprivation of herpes simplex virus infected cells resulted in (*a*) inhibition of the synthesis of infectious virions[1-5]; (*b*) a decline in the rate of cellular protein synthesis[3]; (*c*) a continuous replication of viral DNA[3]; (*d*) synthesis of virus specific proteins[6]; and (*e*) synthesis of

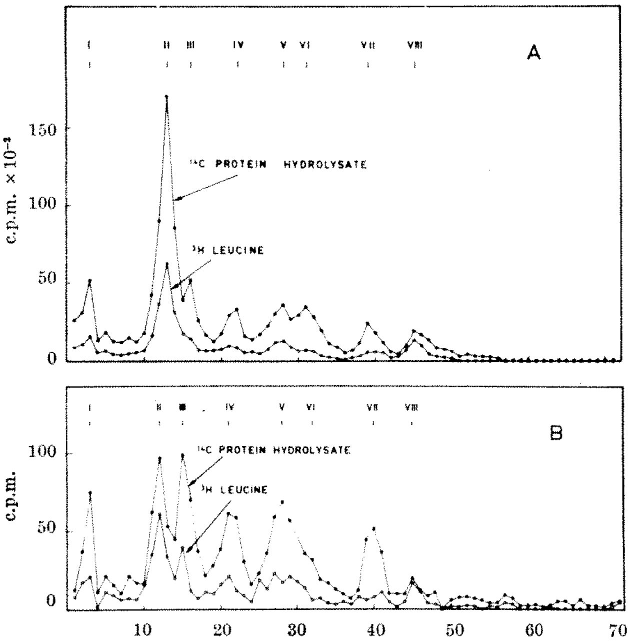

Fig. 1. Electrophoretograms of proteins in nuclear (*A*) and cytoplasmic (*B*) herpes simplex virions, synthesized in the absence and presence of arginine in the culture medium. BSC$_1$ cells were infected with 10 p.f.u./cell of the HF strain of herpes simplex virus. After an adsorption period of 3 h at 37° C, the cells were washed and incubated for 15 h in Eagle's medium without arginine but containing 1/40 the amount of leucine and 10 μCi of ^{3}H-leucine. At the end of this period, the infected cell cultures were washed with buffer and reincubated for 5 h in medium containing excess leucine (10 times the regular concentration), arginine (twice the regular concentration) and 1/40 the concentration of all the other essential amino-acids. The cultures were labelled with 10 μCi of ^{14}C-algal protein hydrolysate. At the end of the incubation period the cells were scraped off the glass and disrupted in a Dounce glass homogenizer. The nuclear and cytoplasmic fractions were obtained and the enveloped virions were prepared by centrifugation in sucrose gradients. The virus bands were removed from the sucrose gradients, the virions were sedimented at 100,000*g* for 60 min and dissolved in urea, sodium dodecyl sulphate and 2-mercaptoethanol and analysed by electrophoresis in acrylamide gels.

virus specific antigens (ref. 7 and personal communication of A. B. Sabin). Addition of arginine to the deprived cells resulted in the restoration of virus replication and the envelopment of the viral DNA[3]. Recently, the analyses of enveloped herpes virions[8,9] by acrylamide gel electrophoresis[10] demonstrated that the herpes virion is composed of seven major structural viral proteins (designated II to VIII[10]). Two of these proteins (designated II and VIII) constitute the viral capsid; two proteins constitute the viral core proteins (VII and VI); and three glycoproteins (designated III, IV, V) are present in the viral envelopes. Protein III is found in the large outer envelope of the cytoplasmic herpes simplex virions[10]. Protein I seems to be an aggregate of one of the envelope proteins and protein IX constitutes only 1 per cent of the structural proteins[10]. It was therefore possible to identify the virus specific structural proteins synthesized in the infected cells in the absence of arginine and used for virion formation on the addition of arginine.

We have incubated herpes virus infected cells in the absence of arginine and the proteins synthesized were labelled with ^{3}H-leucine. In order to determine if functional viral structural proteins were synthesized in the arginine deprived cells, the radioactive leucine was "chased" with excess leucine, virus synthesis was triggered by adding excess arginine and the newly synthesized viral proteins were labelled with ^{14}C-amino-acids. The analysis of the mature herpes virions demonstrated that the two capsid proteins (designated II and VIII) and the envelope proteins were found to be synthesized in the arginine deprived cells and utilized for the formation of virus progeny when arginine became available.

In order to characterize the structural proteins synthesized in the infected cells in the absence of arginine, BSC$_1$ cells were infected with the HF strain of herpes simplex virus (multiplicity of infection 10 p.f.u./cell). After the initial 3 h of virus adsorption[11], the cells were incubated for 15 h at 37° C in arginine free Eagle's medium[12] containing 1/40 of the regular leucine concentration, as well as 10 μCi per culture of ^{3}H-leucine (specific activity 380 mCi/mmole). At the end of the incubation period, the cultures were washed with buffer and were reincubated at 37° C in Eagle's medium containing excess leucine (ten times) and arginine (twice the regular concentration), as well as 1/40 of the other essential amino-acids. In these conditions further incorporation of radioactive leucine was prevented and the synthesis of virus progeny was resumed. The cells were then labelled with 10 μCi/culture of ^{14}C-algal protein hydrolysate (specific activity 54 mCi/m atom carbon) for a period of 5 h at 37° C. Isotopes were obtained from the Radiochemical Centre, Amersham. The cells were harvested and disrupted in a glass Dounce homogenizer. The nuclear and the cytoplasmic fractions were obtained by centrifugation and the enveloped virions were isolated by zone centrifugation in 12–52 per cent sucrose gradients[8,9]. The bands of enveloped nuclear and cytoplasmic virions were removed from the sucrose gradients and analysed by electrophoresis in acrylamide gels[10,13]. The structural proteins which were synthesized in the infected cells during the period of arginine deprivation were labelled with ^{3}H-leucine. Viral proteins synthesized in the infected cells after the addition of arginine were labelled with ^{14}C-amino-acids.

Previous studies[3] had demonstrated that in the absence of arginine from the culture medium, the synthesis of herpes virions was inhibited, but the addition of arginine to the culture medium resulted in a prompt resumption of virion formation. In the present experiments it was found that the addition of arginine to the deprived cultures resulted in the synthesis of a virus progeny and the herpes virions were recovered from the nuclear and the cytoplasmic fractions of the infected cells. The electrophoretograms of the nuclear virions (Fig. 1*A*) demonstrated the presence of the seven major proteins (II to VIII), all of them labelled with ^{14}C-amino-acids. Two of the proteins (II and VIII), however, also contained protein molecules which were labelled with ^{3}H-leucine. Protein V also contained a small amount of ^{3}H-leucine labelled molecules (Fig. 1*A*). This result suggested that polypeptide chains of proteins II, VIII and possibly also V were synthesized in the arginine deprived herpes virus infected cells, at a time when the formation of herpes virions was completely prevented. These peptides were transported to the nuclei and were utilized for the formation of the virus particles after arginine was added to the cultures. In contrast, the virus progeny contained viral core proteins (VII and VI) as well as the glycoproteins (III, IV and V) which were synthesized after the addition of arginine to the deprived cells. Nevertheless, to determine if protein III (the glycoprotein present in the large viral envelope) was synthesized in the absence of arginine, analyses were also made on herpes simplex virions present in the cytoplasm. We have found (our unpublished results) that the cytoplasmic herpes virions contain more of protein III than the nuclear virions. The results in Fig. 1*B* demonstrate that in addition to tritium labelled proteins II, VIII and V, tritium labelled proteins III and IV were also present in small amounts in the virions, indicating their synthesis in the arginine deprived cells, and were used for the envelopment of the virus particles after the restoration of arginine.

The ability of the arginine deprived herpes virus infected cells to synthesize the viral capsid proteins II and VIII and possibly also the envelope protein V might reflect the amino-acid composition of the proteins. We have found[10] that proteins II, VIII and V have the lowest arginine/leucine ratio among the herpes virus structural proteins (0·74, 0·66 and 0·89, respectively).

This does not explain, however, the synthesis of proteins III and IV which have a higher arginine/leucine ratio. This finding of continued synthesis of proteins II, VIII, IV and V in the herpes virus infected cells, in the absence of an exogenous arginine source, indicates that arginine is available for protein synthesis in the deprived cells, but most probably in restricted amounts. Our finding that capsid and envelope proteins are synthesized in the arginine deprived cells might explain the reports (ref. 7 and personal communication of A. B. Sabin) that herpes viral antigens were synthesized and present in the cytoplasm of herpes virus infected, arginine deprived cells[7]. This might resemble the continued synthesis of adenovirus antigens in arginine deprived adenovirus infected cells[14].

The results might also explain the reason for the lack of herpes virion synthesis in the arginine deprived cells. In these conditions, the synthesis of the virus core proteins (designated VII and VI) or their use and the formation of viral capsids were prevented. It is therefore assumed that in the arginine deprived cells, the packing of the viral DNA was prevented, although the replication of viral DNA was not affected[3]. Thus in the absence of arginine in the medium, the proteins essential for the initial encapsidation of the herpes virus DNA genomes are not available. The synthesis of capsid and envelope proteins will continue in the arginine deprived cells although the formation of virus progeny is inhibited.

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Two Dimensional Cell Separation: Comparison of Embryonic and Adult Haemopoietic Stem Cells

EMBRYONIC and adult haemopoietic stem cells have been shown to differ in such properties as proliferative capacity[1,2], sensitivity to gamma irradiation[3], suicidal levels of tritiated thymidine[4] and seeding efficiency to the spleen[5] as well as susceptibility to suppression of erythroid differentiation in polycythaemic recipients[6].

Moore *et al.*, using the technique of equilibrium density gradient centrifugation, have determined the density distribution profiles of haemopoietic stem cells at various stages of mouse foetal liver development and in adult marrow[7].

The results of this study are summarized in part in Fig. 1, which shows the density distribution profiles of the earliest detectable foetal liver stem cell population (10·5 days gestation) contrasted with that of adult bone marrow. A homogeneous density population of 10·5 day foetal liver stem cells, restricted to the light density region of the gradient, was observed, contrasting with the heterogeneous adult marrow profile located chiefly in the denser regions of the gradient. The density profiles determined at stages of liver development between 10·5 days gestation and birth revealed a progressive shift to the more dense region of the gradient with the appearance of stem cells possessing similar density and spleen seeding efficiency to those of adult marrow.

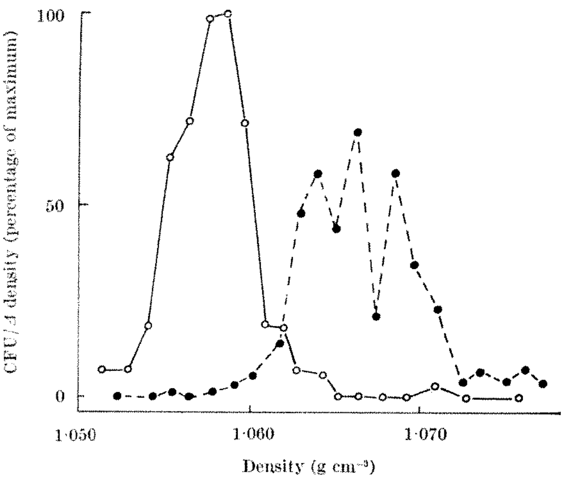

Fig. 1. Density distribution profiles of *in vivo* CFU present in 10·5 day embryonic liver (○—○) and 8 week old adult bone marrow (● - - - ●) from C57Bl–CBA donors. Recipients were given 750 r. X-irradiation. Results were comparable with the C57Bl6 donors used in the present study. The area under the profiles has been normalized for comparison.

Our experiments indicated that only a small proportion $(<2$ per cent) of adult marrow stem cells were of the same density as those of 10·5 day foetal liver. The difference in spleen seeding efficiency of the foetal liver stem cells (4·9 per cent) and stem cells of the same density in adult marrow (12 per cent), however, suggested that density similarity did not imply functional similarity[7,18].

Our purpose here is to describe the application of a further parameter, that of cell size, to characterize more fully stem cells of similar density in early foetal liver and adult marrow. Miller and Phillips[8] have introduced a modified version of the Peterson and Evans method[9] for separation of cells on the basis of differences in cell sedimentation velocity. This sedimentation rate depends largely upon the volume of the cell but also shows a dependence on the density of the cell (unpublished work of J. S. H.) Equilibrium density gradient centrifugation separates cells solely on the basis of density, which is a function of many cell parameters including size[10]. In order, therefore, to obtain the highest degree of resolution among a complex mixture of cells varying in size and density, a separation method using the two techniques in series would be expected to yield much better results than either technique alone.

Stem cells were determined by the *in vivo* CFU assay of Till and McCulloch[11]. The preparation of foetal liver and marrow cells from (CBA × C57)F$_1$ and C57Bl6 mice has been described previously[7]. Because of the density homogeneity (1·0565–1·0585 g cm^{-3}) of 10·5 day foetal liver stem cells, they were not subjected to preliminary fractionation by density. Marrow cells, obtained by flushing femoral shafts of 8 week old mice, were subjected to density gradient separation using the technique of Shortman[10]. In brief, marrow (five donors) or liver cells (twenty-five donors, 200 CFU) were dispersed in a linear gradient of bovine plasma albumin (14–28 per cent by weight) and cells were brought to the position of their buoyant density by centrifugation for 45 min at 4° C. Density fractions were collected by upward displacement of the gradient and, after density determination of each fraction, cells in the density range 1·0565–1·0585 (680 CFU) were pooled and then subjected to a velocity sedimentation separation using the technique of Miller and Phillips[8]. Sedimentation was carried out at 4° C in gradients of

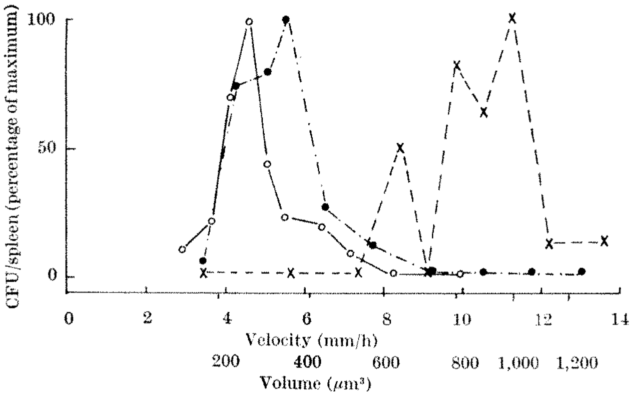

Fig. 2. Sedimentation velocity profiles of *in vivo* CFU present in 10·5 day embryonic liver (× - - - ×), adult bone marrow (ρ1·0565–1·0585) (○—○) and spleen (●—·—●) from lethally irradiated recipients given 2×10^5 adult bone marrow cells 7 days before death. Cell loading in all experiments was less than 3×10^6 cells per ml. (10 ml. sample) for bone marrow or spleen and 2×10^6 cells per ml. for embryonic liver. Cell volumes were determined on the pulse height analyser.

4–30 per cent foetal calf serum in Tris buffered Eagle's MEM (Difco) for 2·5 h in the case of embryonic cells or 4 h in the case of the bone marrow cells. Recovery of CFU activity was 80–100 per cent.

The sedimentation profiles of 10·5 day foetal liver stem cells and of the equivalent density region of adult marrow stem cells are shown in Fig. 2. The results show that the foetal liver stem cells sediment more rapidly than those of equivalent density in adult marrow. Because the foetal and adult stem cells have been selected to possess similar density, the sedimentation velocity results can be directly interpreted in terms of the volume of each cell (unpublished work of J. S. H.). In both of these experiments enrichment of CFU was about ten-fold.

Using a pulse height analyser equipped with a Coulter counter aperture[8], data for the relationship of volume and density with sedimentation velocity were obtained using a range of densities of normal spleen cells (unpublished work of J. S. H.). These data have been used to plot the volumes indicated in Fig. 2. The adult marrow stem cells in density region 1·0565–1·0585 showed a major population of volume 260 μm^3 and a minor population of 430 μm^3 (equivalent to spherical diameters of 7·9 and 9·3 μm). In contrast the foetal stem cells of similar density revealed major populations of volumes 820 and 1,000 μm^3 (equivalent to spherical diameters of 11·6 and 12·4 μm). It is difficult to relate these spherical diameters to values measured on smearing; but mouse myeloma cells of spherical diameter 13 μm have a diameter of 16·5 μm on smearing, so it seems possible that the largest embryonic stem cell would be 15–16 μm on a smear.

Our results indicate that stem cells of early embryonic type are absent from adult resting marrow. It is therefore unlikely that any morphological similarity would be evident. In this context M. A. S. M. and Owen[12,13], using autoradiographic and histological techniques in conjunction with chromosome marker studies, have tentatively described the stem cell in early embryonic haemopoiesis as a large (> 15 μm diameter) blast-type cell characterized by a prominent nucleolus and extensive cytoplasmic basophilia. In contrast, in adult marrow, smaller cells of the small and medium lymphocyte[14], transitional cell[15] or monocytoid[16] types have been implicated as haemopoietic stem cells.

Niewisch *et al.* carried out density separation of adult regenerating spleen and suggested that a minor population of stem cells from exceptionally high density region of the gradient (1·020) were large cells of 14–17 μm diameter in smears and with intensity basophilic cytoplasm[17]. It therefore seemed possible that differences in size and morphology between embryonic and adult marrow stem cells may be the result chiefly of differences in proliferative activity of the stem cell population rather than of a maturation sequence involving irreversible changes in stem cell morphology. In order to test this hypothesis, bone marrow cells (2×10^5 per recipient) were passaged in lethally irradiated recipients for 7 days. A fraction of the spleens of these animals (1,200 CFU) was then submitted to a sedimentation velocity separation for CFU. The results indicated in Fig. 2 are clearly in accord with the notion that adult bone marrow is incapable of reverting to the early type embryonic stem cells, even in conditions of extreme proliferation. It therefore seems that embryonic stem cells undergo a process of differentiation with age which is reflected in volume and density changes.

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Viability of Isolated Nuclei

Amoeba proteus has been used with some success in studies of nuclear and cytoplasmic activities where transfer of the nuclei from one cell to another, or comparative studies of anucleate and nucleate cells, allow separation of the activities[1,2]. This technique, however, answers many questions only partially, for it has been impossible to observe the behaviour of nuclei in the absence of cytoplasm. We report here an initial investigation to obtain a medium in which nuclei can be isolated for short periods without damage.

The ultimate criterion for damage is whether such nuclei can assume their normal functions on return to anucleate cytoplasm; functions, that is, which lead to growth and division of the cell. We decided to use 35 mM KCl as an isolating medium because this is the concentration of K^+, the main intracellular cation[3,4], a fact that previous studies had overlooked[5,6]. Though nuclei could survive in 35 mM NaCl or in the more complex mixtures known to preserve synthetic ability, such as those of Honda[7] and Rusch[8], in our hands KCl has given the most promising results. Isolation of nuclei in 35 mM KCl for periods of up to 15 min gave a survival for nuclei only

Table 1. SURVIVAL OF AMOEBAE CONTAINING NUCLEI ISOLATED IN 35 mM KCl FOR VARYING TIMES

	Controls		Nuclei returned to anucleate cytoplasm after isolation					
	Direct transfer of nuclei from one amoeba to another	Double transfer (retransfer of nuclei 0·25–1 h after the first transfer)	In Chalkley's medium for 5 minutes	In KCl medium for 5–10 minutes	10–15	15–25	40–60	In KCl medium for 7–15 min using the pipette isolating method
No. of transfers	50	30	20	222				65
Percentage viable*	90	70	0	65	60	35	10	25
Average time (days) of first division after transfer	2	3·5	—	7	9	9	14	10

* Operated amoebae were kept in small dishes until they either died or formed a clone of 20–30 cells; they were then moved to vials with wheat grains and kept until they formed clones of several hundred amoebae. This allowed for delayed lethality or for late development of any abnormalities.

slightly lower than that obtained for control double nuclear transfers, that is, 60–65 per cent (Table 1).

The behaviour of an anucleate amoeba given a nucleus which has been isolated separates into three phases.

(1) Reactivation. Amoebae were able to attach to the substratum and move about after 90 per cent of the nuclear operations, provided that the operations themselves had been successful, that is, if the nucleus had been pushed clear of the cytoplasmic membrane and the tear in the membrane repaired. Attachment occurred within 15 to 30 min of the operation, but unless it was followed by acceptance of the nucleus the ability to attach and move disappeared after one to several days and amoebae then behaved as if they contained no nucleus. Some factors influenced the speed of reactivation; for example, young isolated nuclei were good reactivators, anucleate cytoplasm given 1–2 h old nuclei attaching sooner than cytoplasm with nuclei of other ages; nuclei approaching division were poor reactivators, some 8–12 h frequently passing before amoebae became attached and moved; nuclei isolated for periods of 20 min or more reactivated, but took several hours to do so. About 30 per cent of reactivated amoebae did not assume any activities other than attachment and movement.

(2) Acceptance. When attachment and movement continued for several days and were accompanied by feeding, growth and continued survival, it was assumed that the nucleus had been accepted by the cell. About 10 per cent of these nuclei were able to assume only a limited number of nuclear activities. Such cells died without dividing 3–6 weeks after the transfer operation.

(3) Division. The ability of operated amoebae to form viable clones of apparently normal amoebae was taken as proof that such nuclei received no irreversible damage during their period of isolation. Sixty per cent of reactivated amoebae were capable of division. Almost all such amoebae continued to divide and formed apparently normal clones.

Many factors, such as the age of the nucleus at the time of isolation, contributed to the success of isolated nuclear operations. Nuclei isolated when less than 3 h old stuck to the glass operating needle, were very difficult to see or to move and, even when successfully transferred and accepted by anucleate cytoplasm, were usually unable to divide. Nuclei approaching division at the time of isolation frequently underwent abnormal divisions. The percentage of nuclei accepted by anucleate cytoplasm was greatly influenced by the operation procedure. The most successful method of isolation involved the use of the oil chamber nuclear transfer method developed by de Fonbrune with donor amoebae in drops of KCl, recipient enucleated amoebae in small drops of Chalkley's medium and a second larger drop of Chalkley's medium placed nearby into which operated amoebae could be drawn immediately the operation was over. Large differences in survival occurred if, for example, recipient cytoplasm was left for any length of time in the KCl medium; if more than one amoeba was allowed to cytolyse in the drop containing isolated nuclei; or if more than a very small amount of Chalkley's medium was allowed to accompany the recipient cytoplasm into the drop of KCl with isolated nuclei.

Nuclei used for *in vitro* studies have to be moved from one drop to another. To simulate these conditions nuclei were isolated in KCl in small dishes under a dissecting microscope by sucking amoebae into and out of a narrow pipette. Nuclei could be picked up as they floated free of bursting amoebae, and washed in fresh KCl. The final stage of the operation was completed with the de Fonbrune manipulator after placing the nuclei in drops of KCl in oil chambers. Exposures of 7–15 min using nuclei isolated in this way have so far given a much lower survival than was obtained when nuclei were handled only during the moments of isolation and retransfer. Attempts are being made to improve this technique, both for use in treating isolated nuclei with chemicals and for following the activities of isolated nuclei using labelled nucleic acid and protein precursors, followed by reintroduction into anucleate cells.

Previous experiments on *Amoeba proteus* have shown that nuclei are seldom able to survive more than 1 min isolation in a variety of saline and sugar media[5,6]. Our experiments show that nuclei can survive much longer periods of isolation without permanent damage if a 35 mM KCl medium is used. Immediate changes to the nucleus, however, such as shrinkage and loss of turgidity, take place while nuclei are in KCl. Such changes are evident for several days after retransfer, the shrivelled nuclei swelling in the recipient cytoplasm but remaining soft and transparent. Use of tritium-labelled uridine showed that no measurable amount of DNA transcription took place at this stage, and several days to more than a week elapsed before growth and division of the amoebae occurred (Table 1).

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Isolation of Anti-polysaccharide Antibodies with Glutaraldehyde Cross-linked Lipopolysaccharides

IMMUNOADSORBANTS are used chiefly for the isolation of antibodies against protein antigens. Glutaraldehyde has already been successfully used as a cross-linking agent to convert carboxypeptidase A[1], trypsin[2] and protein antigens[3] into insoluble derivatives retaining their specific biological properties. Few attempts have been made to

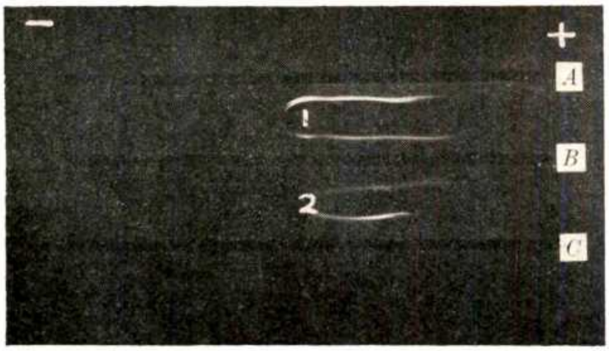

Fig. 1. Immunoelectrophoresis of lipopolysaccharide developed against antilipopolysaccharide antibody eluate and whole anti-live-culture serum against *E. coli* serotype 792. Wells (1 and 2): Lipopolysaccharide from *E. coli* serotype 792; troughs (*A* and *C*): anti-live-culture serum against *E. coli* serotype 792; *B*, antibody eluate.

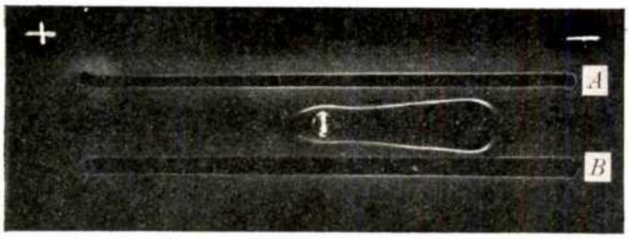

Fig. 2. Immunoelectrophoretic analysis of antibody eluted, developed against donkey anti-rabbit IgG serum. Well: antibody eluate from anti-live-culture serum against *E. coli* serotype 792. Troughs: donkey anti-rabbit IgG serum.

prepare immunoadsorbents from substances other than proteins[4,5]. In so far as lipopolysaccharides have not been insolubilized, we have attempted their cross-linkage to develop a cross-linking procedure, using glutaraldehyde.

The lipopolysaccharide was obtained from a local strain of serotype 792 (classified by Edwards and Ewing[6] as *Escherichia coli* 028 K73 (B), Katwijk type) according to the method of Westphal *et al.*[7]. Glutaraldehyde (final concentration 2 per cent) was added to a 1 per cent solution of lipopolysaccharide in 0·1 M phosphate buffer, *p*H 7·0. The solution was gently stirred for 1 h at room temperature. An equal volume of saturated ammonium sulphate was added and stirring was continued for another 30 min. The resulting precipitate was collected by centrifugation, repeatedly washed with 0·1 M phosphate buffer (*p*H 7·0), suspended in phosphate-buffered saline and stored at 4° C.

A sample containing 14 mg (estimated dry weight) of the suspension was mixed with 1 ml. anti-live-culture serum against serotype 792. The suspension was shaken for 2 h at room temperature. Following adsorption, the serum had an agglutination titre of 1 : 80 whereas the value before adsorption was 1 : 1,600 as determined by the "doubling-dilution" technique; roughly estimated, this meant an adsorption of 95 per cent. The precipitate was washed with phosphate-buffered saline free of protein (until zero absorbance at 280 nm). The bound antibodies were eluted with 0·2 M glycine buffer (*p*H 2·2) in the cold. The antibody recovery amounted roughly to 10 per cent (agglutination titre of the eluate, 1 : 160). This is in agreement with the findings of Kochwa *et al.*[8]. Immunochemical analysis revealed the presence of antilipopolysaccharide antibodies in the eluates (Fig. 1). The immunoelectrophoretogram (Fig. 2) shows that they belong to the IgG class but contain small amounts of IgM and also traces of albumin.

The lipopolysaccharide condensate was stored in the cold after washing with phosphate-buffered saline. Its O-specific determinants do not seem to have been damaged by interaction with glutaraldehyde, for it combines specifically with homologous antibody and not with heterologous antibody against *E. coli* 025. The immunoadsorbant was used repeatedly (four times) without significant reduction in its antibody binding capacity.

The mechanism of lipopolysaccharide–glutaraldehyde aggregation is not understood. Further experiments to elucidate the nature of the reaction and to improve quantitative antibody recovery are in progress.

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Two Fractions with Macrophage Migration Inhibitory Activity from Sensitized Lymphocyte Cultures

Landsteiner and Chase[1] established the cellular basis of delayed hypersensitivity when they showed that the passive transfer of the delayed-type reaction from sensitized guinea-pigs to normal animals could be achieved with peritoneal exudate but not with serum. This immunological response can be measured *in vitro*, for the migration of non-sensitized macrophages can be inhibited by a migration inhibitory factor (MIF) produced by sensitized lymphocytes cultured in the presence of specific antigens[2,3]. Intradermal injection of MIF into non-sensitized guinea-pigs produces reactions characterized by induration, erythema and mononuclear infiltration.

Lymphocytes were obtained from the axillary, popliteal and inguinal lymph nodes of Hartley guinea-pigs which had been sensitized with complete Freund's adjuvant. A single cell suspension was prepared and cultured for 24 h at 37° C in the presence of 50 μg/ml. of tuberculin purified protein derivative (PPD; Parke Davis) in Eagle's minimum essential medium (MEM) containing 50 units penicillin and 50 μg streptomycin per ml. The cell suspension was centrifuged and the cell-free supernatant freeze dried and stored at −20° C. Sensitized cells cultured without added PPD were used as controls: 50 μg/ml. PPD was added just before lyophilization. In a typical experiment, 60 ml. of cell-free supernatant was lyophilized and the residue reconstituted with distilled water applied to a column (2·5 × 120 cm) of 'Sephadex *G*-100' which was equilibrated and eluted with 0·02 M Tris–HCl–0·1 M NaCl buffer, *p*H 7·3. Absorbance was measured at 280 nm and the effluent was separated into five fractions each of which was concentrated to 3 ml. Foetal calf serum (15 per cent) was added to aliquots of each fraction which were then tested in cell migration chambers as previously described[3]. Only fractions II and V were found clearly to inhibit the migration of macrophages. The MIF activity in fraction V cannot be simply attributed to a non-specific cytotoxic substance, for there was no detectable cytotoxic effect on non-sensitized guinea-pig lymphocytes as evaluated by ^{51}Cr release; rabbit anti-guinea-pig lymphocyte serum used in the same assay effected significant ^{51}Cr release.

Polyacrylamide gel electrophoresis[4] provided good resolution which revealed differences in the electrophoretic patterns of the five 'Sephadex' fractions. It was not possible, however, to ascribe MIF activity to a single electrophoretic component. The actual amount of MIF is extremely small because fraction II, on electrophoresis, had its activity contained only within 15 μg protein distributed among several electrophoretic components. This amount represents about 3 per cent of the protein (530 μg) applied to the gel. In terms of the total protein applied to the 'Sephadex' column (2·5 mg), MIF present in fractions II and V was purified approximately 150-fold and 60-fold, respectively. These fractions have average molecular weights of 67,000 and 12,000 respectively, as estimated from their elution positions on a calibrated 'Sephadex *G*-100' column. Bennet and Bloom[5] have previously estimated that MIF has a molecular weight of 67,000 and is able to induce in normal guinea-pigs inflammatory reactions which resemble delayed type reactions. Our fraction II seems to be quite similar to the MIF described by these investigators: it also produced a significant skin reaction in non-sensitized guinea-pigs. By contrast, fraction V exhibited *in vitro* activity, but no detectable ability to induce inflammatory reactions. Fraction V is not simply attributable to PPD, for in control experiments no MIF activity was found in its elution position on 'Sephadex *G*-100'.

The lack of skin reactivity of fraction V (mol. wt. 12,000) could be ascribed to its relatively small molecular size which, in turn, could cause more rapid diffusion from the reaction site and thus make this fraction less effective in the induction of the inflammatory reaction. Our data provide some evidence that the MIF in fraction II may be a different molecular species from that found in fraction V. Consequently, MIF might be a relatively small molecule which is able to associate with carrier molecules of various sizes. This would provide a mechanism for MIF to remain in the reaction site long enough to stimulate the inflammatory reaction.

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Macrophage Migration Inhibition Test using Guinea-pig Macrophages and Human Lymphocytes

The capillary macrophage migration inhibition test[1,2], one of the few available satisfactory methods for the *in vitro* study of delayed hypersensitivity, has largely remained an animal experimental technique, for a ready source of human macrophages has not been available. The usefulness of the method has thus been limited. Attempts by Lawrence and David[3] to use human blood cells were fraught with technical difficulties and therefore apparently abandoned. The subsequent isolation of migration inhibition factor[4,5] diverted attention to the study of its properties. For example, when the factor was obtained from sensitive human lymphocytes it could inhibit guinea-pig macrophages[6], but exploitation of this property did not increase significantly the study of human delayed hypersensitivity. The absence of sufficient macrophages (monocytes) in human peripheral blood for the test to be carried out successfully was overcome by culturing lymph node cells for 72 h and then performing the usual capillary migration test[7]. None of these modifications has had wide application because of numerous practical difficulties brought on by further complication of an already difficult technique. The case of peripheral blood cells[8] failed to stand up to critical testing[9]. We wish to make a preliminary report of a successful method for the capillary migration inhibition test using guinea-pig macrophages and human peripheral blood lymphocytes. Fuller details of the technique, in view of its possible wide application in the study of human disease, will be published later.

Macrophages and lymphocytes were prepared separately for each experiment. The cells were mixed and, where appropriate, antigens were added in varying strengths. Capillary tubes were filled and placed in the incubation chambers.

To obtain the macrophages four or five cross-bred guinea-pigs of the Hartley strain were injected with 25–30 ml. of sterile warm liquid paraffin. Five days later the macrophages were washed out of the peritoneal cavity aseptically with N Parker 199 and pooled. The peritoneal exudate cells were separated, spun and washed three times in N Parker 199. The second wash was preceded by osmotic lysis of red cells with 0·5 N Parker 199 for exactly 60 s and the osmolality was corrected by the addition of 1·5 N Parker 199. To obtain the lymphocytes 25 ml. of peripheral venous blood from human volunteers was defibrinated with glass beads and mixed with 3 per cent gelatine[10]. After 45–60 min the supernatant containing the white blood cells was collected, spun and washed three times with N Parker 199. The second wash was preceded by osmotic lysis of red blood cells as for the macrophages. The lymphocytes were then counted.

Twelve conical centrifuge tubes each containing 20×10^6 macrophages were arranged in four rows of three. The tubes of the first row were to contain no antigen, those of the second row 1 μg of PPD (purified protein derivative of tuberculin) per ml., the third row 10 μg of PPD/ml. and the fourth row 100 μg of PPD/ml. In the first column all four tubes contained macrophages but no lymphocytes. In the second and third columns lymphocytes were added to a level of 3 per cent and 15 per cent respectively relative to the macrophages.

The contents of the tubes were mixed gently, spun at 800 r.p.m. in an MSE bench centrifuge, drained and 0·5 ml. of fresh medium (N Parker 199 containing 200 IU/ml. of penicillin and 100 μg/ml. of streptomycin, 15 per cent foetal calf serum and 4 per cent by volume of 4·4 per cent $NaHCO_3$) or medium containing PPD as mentioned before was added. The packed cells were resuspended, and capillary tubes were filled, spun and cut, and the cell-containing portions were placed in chambers as described by David[2]. The chambers contained the same medium and concentrations of PPD as before. Each chamber received two capillary tubes and at least two chambers were used for each concentration of antigen. After the chambers had been incubated at 37° C for 24 h the area of migration was read and expressed as a percentage of the area achieved by the cells in the same column but without added antigen. Table 1 shows the results obtained on five individuals, three tuberculin positive (that is, a Heaf test of 4 or more) and two tuberculin negative. There is a clear cut delineation between the tuberculin positive and negative individuals in the presence of the larger doses of antigen in the presence of both 3 per cent and 15 per cent of lymphocytes.

The first column of tubes which contains no lymphocytes acts as a control for any direct toxicity of the antigen on the migrating macrophages. The first row of tubes reveals any inhibitory effect resulting from the admixture

Table 1. MIGRATION INHIBITION OF GUINEA-PIG MACROPHAGES BY SENSITIZED AND NON-SENSITIZED HUMAN LYMPHOCYTES IN THE PRESENCE OF ANTIGEN

Antigen added (μg/ml. of PPD)	Tuberculin reaction of lymphocyte donor	Macrophages 20×10^6		
		No. lymphocytes (per cent)	3 per cent lymphocytes	15 per cent lymphocytes
0	(*A*) Positive	100	100	100
	(*B*) Positive	100	100	100
	(*C*) Positive	100	100	100
	(*D*) Negative	100	100	100
	(*E*) Negative	100	100	100
1	(*A*) Positive	103*	99·9	112
	(*B*) Positive	74·6	75·1	100·6
	(*C*) Positive	111	79·5	94·0
	(*D*) Negative	96	102	107
	(*E*) Negative	100	107	108
10	(*A*) Positive	85	82	103·7
	(*B*) Positive	105	88·7	98·0
	(*C*) Positive	107	83·5	88·9
	(*D*) Negative	92	100·5	103·5
	(*E*) Negative	102	103	104·5
100	(*A*) Positive	85	44·6	39·4
	(*B*) Positive	98·5	56·8	62·7
	(*C*) Positive	87·8	39·8	54·2
	(*D*) Negative	83·0	92·0	96·0
	(*E*) Negative	92·5	95·0	98·5

* Each reading is the mean of the area of spread in four or six migrations

The reading in each column is the area of migration expressed as a percentage of the area achieved without added antigen. The figures in the first column are a measure of the direct toxicity of antigen on the macrophages.

of cells from two different species. This is not shown in Table 1 in which each migration is shown as 100 per cent for comparison with the other tubes in the same column. It was, however, never sufficiently pronounced to obscure the inhibitory effect of added antigen.

The results presented are merely a small example from a larger study of delayed hypersensitivity to streptococcal and tissue antigens in post-streptococcal states as revealed by migration inhibition.

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Ovotransferrin with One Iron Atom

TRANSFERRINS are iron-binding proteins which can carry a maximum of two atoms of iron per mole protein. If a preparation of hen ovotransferrin is incompletely saturated with iron and subjected to isoelectric focusing three main protein fractions are observed[1]. The most acidic fraction (pH_I 5·78) is the protein complex with two iron atoms, and the most alkaline fraction (pH_I 6·73) is the metal-free protein. In the intermediate fraction (pH_I 6·25) iron and protein are present in equimolar amounts and Wenn and Williams[1] suggested that this fraction is a protein complex with one iron atom.

Woodworth, Tan and Virkaitis[2] suggest, however, that the intermediate isoelectric fraction represents a dimer composed of one molecule each of metal-free protein and iron-saturated protein. According to their interpretation of gel filtration experiments the intermediate isoelectric fraction is a labile dimer and on polyacrylamide gel electrophoresis at pH 7·5 it apparently dissociates, giving two bands which correspond to the metal-free protein and the protein complex with two iron atoms. They state they have never observed an intermediate band corresponding to a protein complex with one iron atom upon polyacrylamide gel electrophoresis.

Evidence against the proposed dimer is provided by the earlier experiments of Stratil[3]. After addition of increasing amounts of iron to whole egg white, starch gel electrophoresis gave an iron-containing band lying between the bands representing the metal-free protein and the iron-saturated protein. The intermediate band disappeared on complete saturation with iron and all the ovotransferrin was then present as the protein complex with two iron atoms. Stratil[3] suggested that the intermediate band consisted of protein molecules bearing only one atom of iron. We now report experiments on the effect of adding iron to purified metal-free ovotransferrin as determined by starch gel electrophoresis and ultracentrifugation.

Ovotransferrin bearing different amounts of iron can be resolved by starch gel electrophoresis if contaminating metals are removed from the starch powder (Connaught hydrolysed starch, lot 270–1) by washing successively with 0·02 M EDTA and deionized water. Dialysis tubing and glassware were cleaned similarly. Iron, as 100 mM iron-nitrilotriacetate[4], was added to a 2 per cent solution of the metal-free protein in 0·05 M $NaHCO_3$. Starch gel electrophoresis at pH 8·6 illustrated (Fig. 1) that in the course of converting the metal-free protein into the fully saturated iron-transferrin complex an intermediate band exists. It seemed likely that this band would correspond to the intermediate fraction observed in isoelectric fractionation experiments[1].

Isoelectric fractionation was therefore performed on a partially iron saturated preparation of ovotransferrin as described previously[1] and the intermediate fraction (pH_I 6·25) was isolated, dialysed against deionized water (NH_4OH added to pH 9) to remove ampholytes and sucrose, and finally lyophilized. The pink coloured powder was dissolved in 0·05 M $NaHCO_3$ and examined by starch gel electrophoresis with markers of metal-free ovotransferrin and iron-saturated ovotransferrin. Fig. 2 demonstrates that the isolated fraction gives one main band whose mobility is intermediate between those of the two marker proteins; it also contains a small amount of the fully saturated protein. The electrophoretic mobility of the intermediate fraction in starch gel suggests that its molecular weight is not twice that of the marker proteins, for it would then be expected to move more slowly.

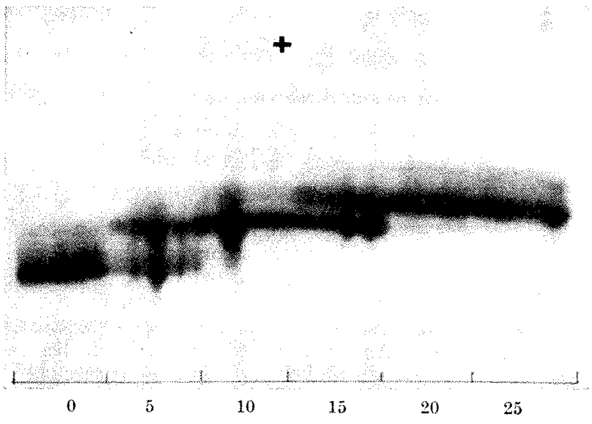

Fig. 1. Starch gel electrophoresis of ovotransferrin in the discontinuous buffer system of Poulik[7]. 11 mg of metal-free ovotransferrin was dissolved in 0·5 ml. 0·05 M $NaHCO_3$. Successive additions of 5 μl. of 100 mM iron-nitrilotriacetate were made and samples were removed for electrophoresis. The addition of 20 μl. of iron-nitrilotriacetate resulted in complete saturation.

In sedimentation experiments, batches of metal-free ovotransferrin dissolved in different buffer solutions

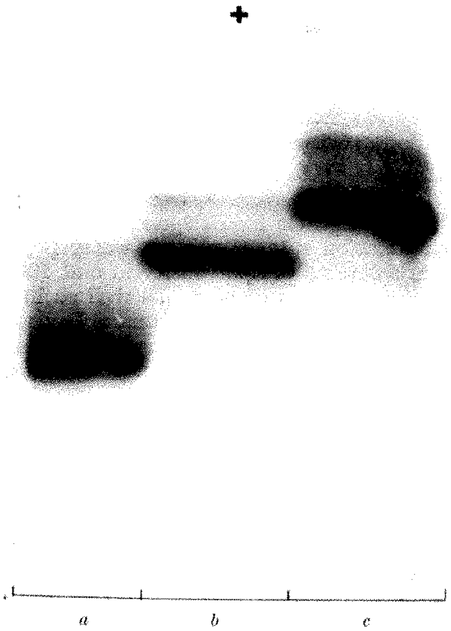

Fig. 2. Starch gel electrophoresis. Samples *a* and *c* are iron-free ovotransferrin and fully iron saturated ovotransferrin, respectively. Sample *b* is the intermediate band recovered from the isoelectric column.

were divided into several aliquots which were then titrated with 100 mM iron-nitrilotriacetate to give different degrees of iron saturation, estimated from the $A_{470\text{ nm}}$. In all cases the peaks were allowed to migrate at least half-way across the cell and they showed no heterogeneity. In the case of the half saturated and wholly saturated protein the absorption pattern of the sedimenting material (as measured with an interference filter at 460 nm) coincided with the peak observed in the Schlieren experiments. In addition, in all experiments the whole of the protein could be accounted for refractively. After removal from the cell the samples showed no increase in iron content, as judged by the $A_{470\text{ nm}}$. The sedimentation coefficients observed in three experiments are given in Table 1. In experiment 2, the buffer was the same as that used for starch gel electrophoresis. There is no increase in the sedimentation coefficient at half saturation which might have suggested the presence of a dimer. In all cases the concentration dependence of the sedimentation coefficient was greater for the fully iron-saturated protein than for the metal-free protein, which agrees with the finding by Fuller and Briggs[5] that iron-transferrin has a greater axial ratio than the metal-free protein.

Table 1. SEDIMENTATION COEFFICIENTS OF OVOTRANSFERRIN SATURATED TO DIFFERENT EXTENTS WITH IRON

Buffer	Per cent iron saturation 0	25	50	75	100
Imidazole-HCl (0·05 M), NaCl (0·05 M), *p*H 6·8	5·1	5·0	4·9	4·8	4·9
Tris-citrate (0·076 M), *p*H 8·6	4·6	—	4·9	—	4·8
Tris (0·075 M), $NaHCO_3$ (0·05 M), HCl, *p*H 8·6	4·8	4·8	4·6	4·5	4·8

The sedimentation coefficients relate to zero protein concentration at 19° in the quoted buffer solutions.

The results of starch gel electrophoresis and ultracentrifugation thus support the earlier proposal[1] that the intermediate fraction observed during isoelectric fractionation is a transferrin complex with one iron atom. This implies that iron atoms are distributed among the metal-binding sites on the protein in an essentially random manner[1,6,8] and it is contrary to the view that the metal atoms bind only in pairs to the protein[4,9,10]. Many authors[1,4,6,8–10] have apparently assumed that a random distribution of metal atoms between the binding sites could only arise if the dissociation constants for the first (K_1) and second (K_2) metal atoms were approximately equal. K_2 has been variously reported to be less than K_1 (ref. 11), approximately equal to K_1 (ref. 6) or much greater than K_1 (ref. 9). It is clear, however, that the binding is effectively irreversible at alkaline *p*H (dissociation constant $\simeq 10^{36}$ M; ref. 6), and it has been shown[12] that no exchange of iron between transferrin molecules occurs at *p*H 7·3 in the absence of chelating agents. We suggest that the iron atoms are randomly distributed on the binding sites, which reflects only the availability of these sites, and we cannot draw conclusions as to the relative values of the dissociation constants.

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Absence of Dimers and Nature of Iron Binding in Transferrin Solutions

A CONTROVERSIAL question in the chemistry of the transferrins is whether the two specific sites of the proteins bind metal ions pairwise or randomly. Equilibrium dialysis studies by Aasa *et al.*[1], in which care was taken to show that true equilibrium had been achieved, indicated that the stability constants for the sites were virtually identical; this suggested that the binding sites were equivalent and non-interacting. This work was corroborated by studies using the techniques of moving boundary electrophoresis[2] and isoelectric focusing[3]. In each of these studies, three species of transferrin were recognized and it was suggested that these represented molecules containing nought, one or two iron atoms. This assignment has been challenged by Woodworth *et al.*[4], who argued that the species with intermediate electrophoretic mobility or intermediate isoelectric point was really a dimer composed of molecules devoid of iron and molecules containing two iron atoms. This alternative interpretation was taken as supporting the earlier contention of Woodworth based on kinetic studies that iron atoms bind to transferrin only pairwise, and that solutions of transferrin less than fully saturated with iron would contain only two separable species of protein molecules, those without any iron and those with two iron atoms per molecule[5].

The existence of dimers in protein solutions is accessible to direct experimental investigation. We have used three independent methods to search for their presence. Because Woodworth *et al.*[4] have argued that dimer formation is facilitated in solutions at lower than neutral *p*H, we have carried out sedimentation equilibrium[6] and sedimentation velocity studies in solutions of transferrin half saturated with iron (added as the nitrilotriacetate

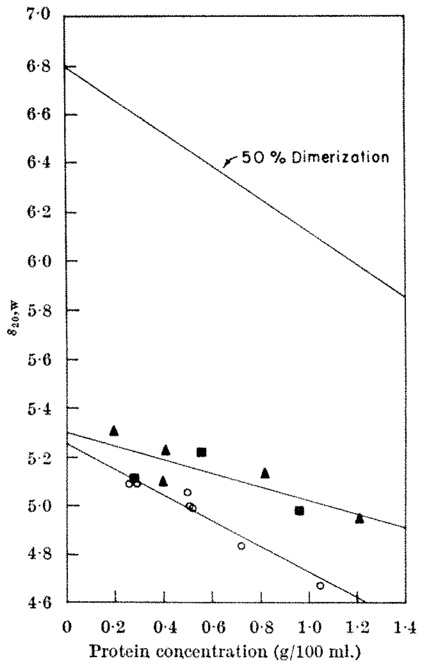

Fig. 1. Sedimentation velocity of apotransferrin in 0·1 M NaCl, *p*H 6·7 (○); 1 : 1 Fe–transferrin in cacodylate buffer, *p*H 6·7, ionic strength 0·1 (■); and 1 : 1 Fe–transferrin in 0·1 M NaCl, *p*H 6·7 (▲). The uppermost line is that calculated for 50 per cent dimerization.

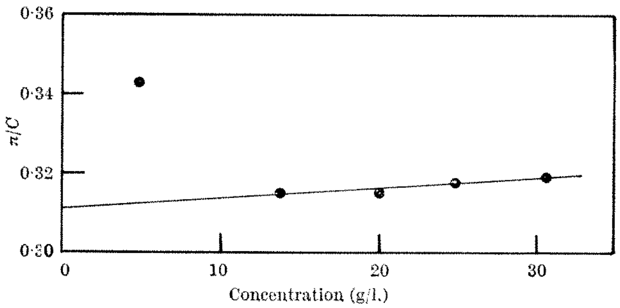

Fig. 2. Osmotic pressure (in cm of saline) in solutions of transferrin 48 per cent saturated with Fe^{3+}, measured by a Hewlett–Packard model 503 high speed membrane osmometer operating at 25° C. The solid line represents a least-squares fit to the data points, omitting the point at lowest concentration which deviates appreciably from the line because of the increased likelihood of experimental error in this region. The number average molecular weight at zero concentration is 80,600 daltons; taking into account the point at lowest concentration would lower this value to 74,700.

complex) in cacodylate and in saline buffers of ionic strength 0·1 and *p*H 6·7. This is the buffer system in which three species of transferrin molecules were recognized by moving boundary electrophoresis; the species of intermediate mobility which Woodworth *et al.*[4] suggested was the dimer form of transferrin was the predominant component at half-saturation. The results of the ultracentrifugal studies are given in Table 1 and Fig. 1. The negative regression in the plot of $s_{20,w}$ against concentration is characteristic of non-aggregating systems. The values of $s^0_{20,w}$ provide additional evidence that no detectable dimer is present. The molecular weight of 78,500 obtained for the half-saturated preparation is that of the monomeric species (K. G. M., W. F., A. C. Cox and C. Tanford, submitted for publication).

Results of osmotic pressure measurements in solutions of transferrin 48 per cent saturated with iron at *p*H 6·2 in 0·15 M NaCl are given in Fig. 2. The molecular weight of 80,600 daltons obtained by extrapolation to zero concentration is in good agreement with the values obtained from sedimentation measurements. Again, there is no evidence indicating the existence of dimers.

Table 1. ULTRACENTRIFUGAL STUDIES OF HUMAN SERUM TRANSFERRIN

Preparation	$s^0_{20,w}$	Molecular weight based on: Sed. velocity*	Molecular weight based on: Sed. equilibrium
Apotransferrin† (0·1 M NaCl, *p*H 6·0)	5·25	77,700	
1 : 1 Fe transferrin (0·1 M NaCl, *p*H 6·7, or 0·1 M sodium cacodylate, *p*H 6·7)	5·30		78,800
2 : 1 Fe–transferrin (0·1 M NaCl, *p*H 6·5)			76,600

* The partial specific volume ($\bar{v}$) for this calculation is taken as 0·725 cm^3/g (K. G. M., W. F., A. C. Cox and C. Tanford, submitted for publication; †, the data on apotransferrin are also taken from this source).

Finally, the magnetic field dependence of the nuclear magnetic relaxation rate of solvent water protons[7] was measured in solutions of apotransferrin and 100 per cent saturated transferrin as controls, as well as in a 50 per cent saturated transferrin solution and a 1 : 1 mixture of the control samples. The circles and crosses in Fig. 3 show the data for the latter two samples. The solid line through the points is the result predicted if no dimerization occurs; it is obtained by averaging results for apotransferrin and fully saturated transferrin (Fig. 1 of ref. 7). The upper solid curve is the result predicted for 100 per cent dimerization, assuming that the amount of water bound per mg of protein does not change, that the accessibility of water to the iron sites does not change, and that the geometric factors that affect the rotatory Brownian motion of the dimer are the same as those for the monomer. Given the magnitude of the difference between the two solid curves, it is highly unlikely that errors in these assumptions would produce a result which agrees with the present data as well as does the curve predicated on the assumption of no dimerization. The curves for transferrin brought to 50 per cent saturation with Fe^{3+} by the simple addition of metal, and equimolar mixtures of apotransferrin and fully saturated transferrin, coincide, with no detectable evidence of dimerization in either.

We therefore conclude that dimer formation does not occur in transferrin solutions at any degree of saturation with iron, and that the existence of three types of transferrin molecules by a moving boundary electrophoresis[3], starch gel electrophoresis[8], and isoelectric focusing[4] reliably indicates that iron atoms do not bind to transferrin molecules in pairwise fashion.

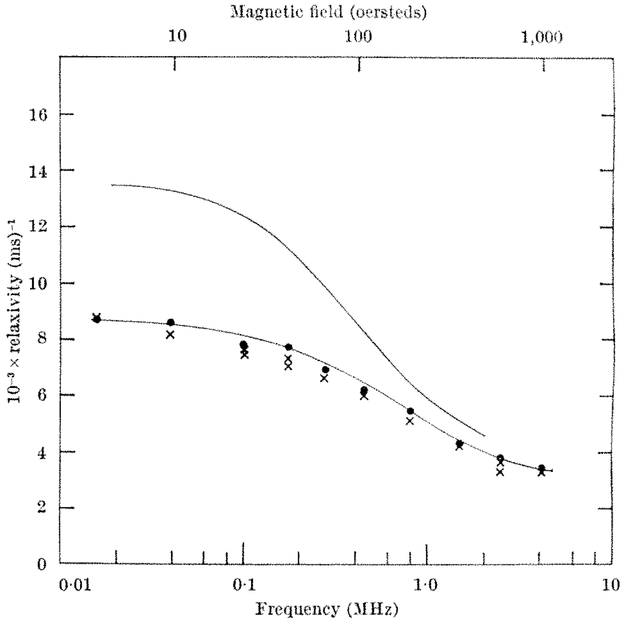

Fig. 3. Nuclear magnetic relaxation dispersion in transferrin solutions. Total protein concentration 4·9 per cent; 0·1 M KCl, *p*H 7·6. Transferrin 50 per cent saturated with Fe^{3+} (●); equimolar mixture of apotransferrin and 100 per cent saturated transferrin (×). The lower solid curve is the result anticipated for both samples in the absence of dimerization. The upper curve is the result anticipated for 100 per cent dimerization for one particular set of assumptions (see text).

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Oxidation of Haemoglobin by Oxygen in Light: Possible Role of Singlet Oxygen

THE transformation of oxyhaemoglobin (HbO_2) to methaemoglobin (Hb^+) involves an electron transfer whereby the ferrous haem iron is oxidized, with the simultaneous reduction of the oxidant, oxygen. In the event of the O_2 being fully reduced (to H_2O) in the process[1], 1 mole of HbO_2 will be expected to release 0·75 mole of O_2, for an electron donated by the ferrous iron can reduce only a quarter of the oxygen released by the iron in going to the trivalent state. In a study of the conversion of oxymyoglobin to metmyoglobin, however, George and Stratmann[2] have reported the consumption of 2·5 moles of O_2 for each mole of haem iron oxidized and attributed the excess to unspecified electron donor groups in the protein moiety.

We have investigated whether the excess oxygen consumption might be linked to a photochemical reaction. We used human HbO_2 in conditions similar to those described by George and Stratmann (*p*H 5·6, 30° C); the test reactions were carried out under moderately strong incident white light, those reacted in darkness serving as blanks. The results indicate that a photodynamic effect is indeed involved; the excess consumption occurred only in light and the dark reaction gave rise to the release of nearly the expected quantity of gas (Fig. 1). The optical absorption spectrum of Hb^+ obtained under illumination exhibited small but significant differences from that of Hb^+ obtained either in the dark or prepared by ferricyanide oxidation. In the aquo spectrum (*p*H 6), two new bands at about 535 nm and 565 nm (low-spin type, Brill and Williams[3]) were evident, with a simultaneous decrease in intensity of the charge transfer bands (630 nm and 500 nm). The Hb^+ obtained by either method was reduced and the oxygen binding properties of each sample were then compared with those of native haemoglobin. The recovered haemoglobins displayed more or less significant differences from the normal protein (diminution of Hill coefficient, increase in oxygen affinity), but the protein obtained from Hb^+ produced in the light always showed the greatest difference.

The chemically active agent seems to be the oxygen molecule bound in HbO_2 at a given instant, as distinct from free O_2 in solution: experiments performed with Hb^+CN^- in identical conditions showed hardly any consumption of oxygen. The bound oxygen in HbO_2 may therefore be somehow activated by light energy absorbed by the haem chromophore. We believe that singlet oxygen may be involved, produced through photosensitization by the haem, according to the reaction

$$\text{Haem}-{}^3O_2 \overset{+h\nu}{\rightleftarrows} {}^1\overset{*}{\text{Haem}}-{}^3O_2 \rightarrow \text{Haem}+{}^1\overset{*}{O}_2$$

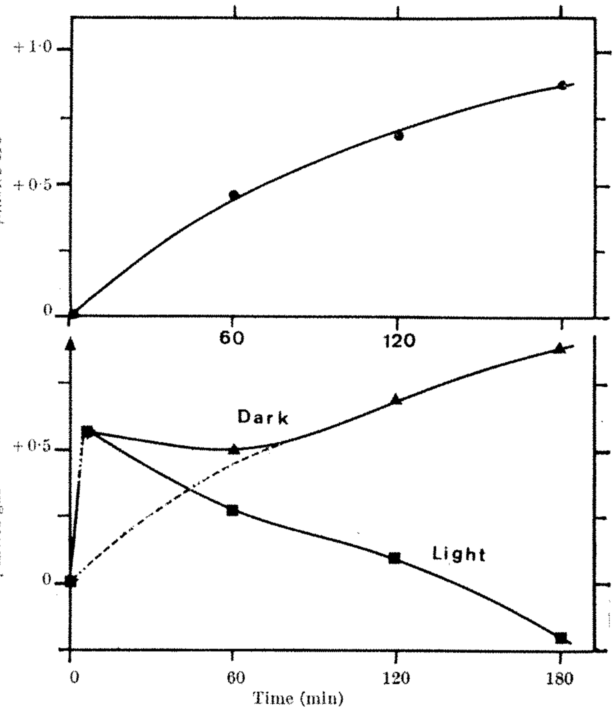

Fig. 1. Top: Net amount of gas evolved or absorbed during the oxidation of HbO_2 in the dark and in light (averaged from three independent experiments). Phosphate buffer (0·65 M, *p*H 5·6), 30° C. Initial concentration of HbO_2, 0·8 mM (haem). Bottom: µmoles gas evolved or absorbed. The initial jump is believed to result from the release of bound CO_2 on lowering the *p*H of oxyhaemoglobin from 6·8 to 5·6. This gas is absorbed by a solution of NaOH placed in the centre well of the Warburg vessel.

Haems are neither fluorescent nor phosphorescent and thus cannot normally sensitize $^3O_2$, but their close proximity to bound O_2 may in some manner compensate for the short lifetime of the excited state. The excited singlet molecular oxygen in its $^1\Delta g$ state is a high energy oxidant with an abnormal lifetime[4], which can act as an energy carrier between a sensitizer and an oxidizable substrate. One may expect long range oxidative action by the continual release of $^1O_2^*(^1\Delta g)$ from its combined state, as is indeed the case in a solution of HbO_2.

Some experimental support for this hypothesis has been obtained from a study of the properties of Hb^+ prepared by the action of chemically generated $^1O_2^*$ on HbO_2. The excited species was generated in a separate compartment ($NaOCl+H_2O_2$) and was transferred to the HbO_2 solution by a stream of nitrogen. The absence of gaseous Cl_2 and the presence of $^1O_2^*$ in the gas stream were ensured by appropriate tests. A solution of HbO_2 treated for a few minutes with $^1O_2^*$ exhibited all the same modified properties as result from the action of oxygen under prolonged illumination. Although this is not unequivocal proof that the active oxidizing agent is identical in each case, it supports the possibility that singlet oxygen, produced by photosensitization through the haem chromophore, is responsible for the extra consumption of oxygen in the oxidation of HbO_2 under illumination.

Chemically generated $^1O_2^*$ might be useful for studying modification in enzymes and other systems, normally achieved through photosensitization[5]—the equivalent of a "photodynamic effect without light".

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Glucose-6-phosphate Dehydrogenase Isoenzymes in Blood Cells

THE enzyme glucose-6-phosphate dehydrogenase (G-6-PD) exists in several different forms (isoenzymes) in the human erythrocyte, each apparently determined by a different allele at a single locus on the *X* chromosome. The structural differences between the isoenzymes lead to different enzymatic activities and often render them separable by gel electrophoresis.

Studies on G-6-PD using enzymatic methods[1] and cytochemical techniques (unpublished results of L. A. R., D. S. and T. Menashe) showed that both normal and G-6-PD deficient erythrocytes of various age groups differ in their enzymatic activity. Because these findings imply that the changes in the enzymatic pattern are a function of age, we decided to check the isoenzyme distribution of G-6-PD in cells of different age groups taken from persons with normal G-6-PD levels.

Heparinized venous blood was used. Thrombocytes and leucocytes were separated by differential centrifugation[2] and the erythrocyte fraction was freed from residual leucocytes by centrifugation and removal of the upper layer. The red blood cells were separated into different age groups according to their density using phthalate esters as the separating medium[3]. The red cells were haemolysed by dilution with aqueous solution of β-mercaptoethanol (1·4 to 14 mM) and/or NADP (0·01 to 1 mM). Leucocytes and thrombocytes were lysed by repeated freezing and thawing. Polyacrylamide gel disc electrophoresis was done using the method of Davis[4] without the sample gel. Staining of the gels to detect G-6-PD activity was performed by the method of Hori *et al.*[5].

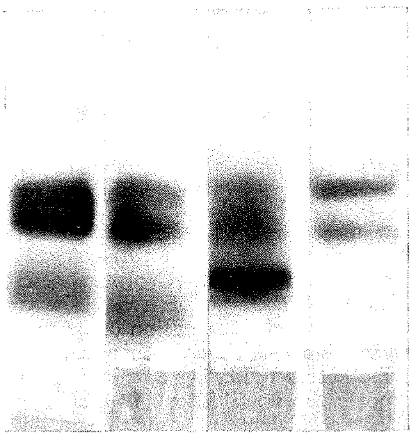

Fig. 1. Polyacrylamide gel disc electrophoresis of red blood cell lysates of different age groups. *a*, Haemolysate of unfractionated erythrocytes; *b*, lysate of "old" red blood cells; *c*, lysate of "young" erythrocytes having three G-6-PD bands; *d*, leucocyte lysate.

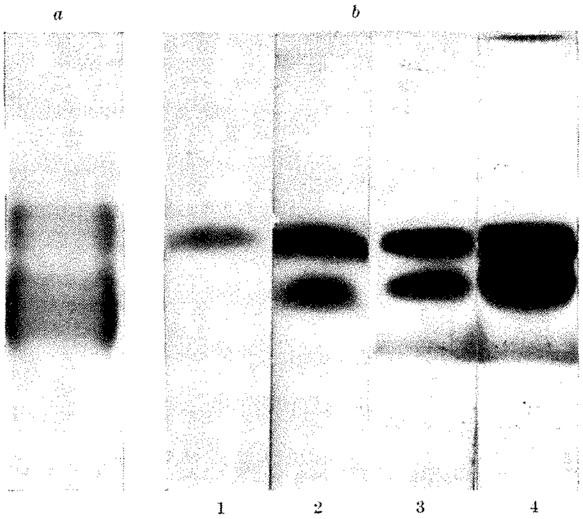

Fig. 2. *a*, Electrophoretic pattern obtained with 0·1 mM NADP added to the gel and upper buffer. Unfractionated erythrocyte lysate was used; *b*, changes in the observed electrophoretic pattern as a function of the amount of applied lysate. Unfractionated lysate was used. 1, 10 μl. lysate; 2, 15 μl. lysate; 3, 20 μl. lysate; 4, 50 μl. lysate.

Sixty-two people were studied, twenty-nine males and thirty-three females, all of whom had normal G-6-PD activity. Disc electrophoresis of the erythrocyte lysates showed a pattern consisting of two enzymatically active bands. The same electrophoretic picture was obtained using haemolysates of "young" cells, "old" cells and unfractionated erythrocytes (Fig. 1). The G-6-PD activity measured spectrophotometrically was, however, higher in the "young" cells than in the "old" ones[1,6].

In most of the cases studied, the band III was the first to appear during staining. This slow band had the same electrophoretic mobility as the haemoglobin A_2 zone. The second band, II, was present in all the cases, but in most of them it seemed to be less active than the slow band III and a longer incubation time was necessary for it to be seen (Fig. 2*b*, 1 and 2). No changes in the electrophoretic pattern were observed between males and females. In three out of the sixty-two cases studied, a third band (I) having G-6-PD activity was visible (Fig. 1*c*) which had a faster electrophoretic mobility and migrated together with the haemoglobin A zone. This band could be found only in the "young" erythrocyte fraction and its enzymatic activity was rather weak compared with the other two active bands. While 90 min incubation in the reaction medium was enough to stain the two slow bands, additional incubation of 12–20 h in water was required before the third band became visible. In all cases, electrophoresis of leucocyte and thrombocyte lysates showed two active bands similar to those found with red cell lysate. No evidence was found for the fast band (I) in these cells.

Application of varying amounts of the different haemolysates to the polyacrylamide gel has shown that an optimal amount of G-6-PD activity should be used (Fig. 2*b*). The use of too small an amount will show the presence of only one band while the use of an excessive amount of activity will cause the two active bands to merge and to appear as one broad band.

Because these electrophoretic patterns were unexpected, it was suspected that they could be electrophoretic artefacts. It was reported[7] that an additional G-6-PD active band could result from disulphide bridge formation between G-6-PD and some serum proteins. Addition of β-mercaptoethanol to the haemolysate, in concentrations ranging from 0·028 M to 0·0028 M, failed to change the electrophoretic pattern, though it should have been sufficient to dissociate any such aggregates. Addition of NADP, in concentrations ranging from 2 mM to 0·02 mM, to the haemolysates, in the presence or in the absence of β-mercaptoethanol, also had no effect on the electrophoretic pattern. When the polyacrylamide gel concentration was varied between 7 and 12 per cent, all G-6-PD active bands were retarded to the same extent, suggesting that all three bands have the same molecular size.

Addition of NADP to the upper buffer and the gel itself has resulted in a further resolution of the bands. The upper band (III) was resolved into two subfractions and the second band (II) was resolved into three (Fig. 2*a*). The presence of NADP was found to increase the sensitivity of the reaction so that small amounts of activity could be detected in this way, which would have been unnoticed in the absence of NADP. The significance of this further separation is under investigation at present. This technique could be used in cases of G-6-PD deficiency where the enzymatic activity is weak and would not be detected by the regular method.

Previous findings[1] that G-6-PD activity declines with ageing of the cell have been confirmed for a particular isoenzyme[1] that is present only in the "young" erythrocytes and absent from more mature cells. This may suggest a change in the enzymatic profile of the cells with ageing, caused by the gradual disappearance of the more labile forms of G-6-PD at an earlier stage.

It has been suggested that in cases of G-6-PD deficiency, there is a point mutation that increases lability and accelerates the rate of destruction of the enzyme[8]. This hypothesis is supported by the finding that in cases of severe G-6-PD deficiency showing no activity in the ordinary spectrophotometric assay, two active bands (Fig. 2*a*) were demonstrated in "young" erythrocytes when NADP was added to the gel and the upper buffer to increase the sensitivity of the reaction.

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Pheromone may induce Brood Tending in the Fire Ant, *Solenopsis saevissima*

OUR observations of laboratory colonies of the fire ant, *Solenopsis saevissima* (F. Smith), suggest that care of the immature forms by the workers might be influenced by secretions from the immature forms. Recognition of the immature forms and their care by workers might thus be associated with these secretions. Watkins and Cole[1] noted that worker army ants, *Neivamyrmex opacithorax* Emery, were attracted to secretions of their larvae and pupae. This letter presents the results of studies we have made on the response of worker fire ants to extracts of their brood.

In the first test, the entire brood from one colony of fire ants was collected, and an extract was prepared from samples of all types of immature forms by homogenizing the pooled forms in cold hexane. The clear extract was then poured over corn cob grits (ground corn cobs, twelve to thirty mesh) which were mixed until the hexane evaporated. The treated and untreated grits were placed on the foraging platform that serves our clear plastic nests. The ants immediately began picking up the treated grits and carrying them to the nest, so in a relatively short time (about 1 h) they had removed all the treated grits and left all the untreated grits. The same response was obtained when clay granules or pieces of paper were treated. Our most important finding, however, was that the ants placed the treated material with their brood within their nest. Later, when we repeated the trials four times with grits, three times with clay granules and three times with pieces of paper, the treated materials were always harvested, but 6 to 24 h later the ants had removed them from the nest to the refuse pile. Ants did not pick up grits treated with hexane alone.

After the preliminary tests, we determined the reactions of the ants to extracts from the various types of immature forms, worker larvae, worker pupae, sex larvae, or sex pupae and to dilutions of these extracts. In this test, 2 g of each form was collected from field colonies and homogenized by hand in a tissue grinder in 2 ml. of hexane. Then the extracts were poured over about two hundred corn cob grits each and evaporated as before. Also, dilutions of 1 : 1, 1 : 10 and/or 1 : 100 of each extract were prepared by appropriate dilution and evaporated on similar numbers of corn cob grits.

The worker ants harvested grits treated with extracts of worker or sex larvae, but they did not harvest any grits treated with extract of worker or sex pupae. When the extract of sex larvae was diluted, workers harvested all grits treated with dilutions of 1 : 1 and 1 : 10 and harvested some grits treated with a dilution of 1 : 100; however, when the extract of worker larvae was diluted, the harvesting of the 1 : 1 dilution was only partial, and no harvesting of grits treated with greater dilutions occurred. Similar results were obtained with ant colonies in soil nests; however, we did not attempt to locate the place where the ants deposited the treated grits in these nests.

In all our studies, the worker ants found and carried the treated grits to their nest, groomed the treated grits, rubbed them with their antennae and palpi, and generally appeared to care for them as they did their brood. In one test, workers carried brood from the nest and placed it with the treated grits on the foraging platform. Moreover, when a colony was disturbed by a sharp tapping that caused an alarm reaction, the worker ants picked up the treated grits and ran about with them in the same manner as they normally do with brood. Workers from subcolonies that had no queen or brood also collected treated grits and returned them to their nest.

Because our method of extraction removed lipids from the brood and because worker ants are greatly attracted to fats and oils, the attraction of the grits that we had noted might be a food response. We therefore tested the response of worker ants in three nests to grits treated with vegetable oil. In two of these colonies, the ants removed the oil from the grits but did not carry the grits into the nests. In the third colony, some grits were carried into the nest, but they were placed apart and separate from the brood. It thus seems that the reaction of the ants to the extract of immature forms was not a food response.

The action of the worker ants of *S. saevissima* in locating, transporting and caring for inanimate objects treated with extracts of brood and placing these treated objects with their brood demonstrated that a pheromone (or pheromones) is associated with recognition and brood tending. The attractant was present in both worker and sex larvae, but not in pupae. This lack of a pheromone(s) in pupae in these preliminary tests does not necessarily preclude its occurrence, however, because we may not have extracted a sufficiently large sample. The removal of the inanimate objects to the refuse piles within 24 h after the introduction into the brood or nests seems to indicate that the pheromone(s) had dissipated.

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Function of the Gravity Receptor of *Octopus vulgaris*

MICROANATOMICAL[1-4] and electrophysiological[5-8] investigations support the hypothesis that the sensory elements of the statocysts of Crustacea and of the vertebrate vestibular endorgans are stimulated adequately by lateral displacement (shear) exerted by the statolith or statoconial membrane. Behavioural findings agree with this kind of statocyst stimulation; for example, equilibrium reactions of fish[9] and Crustacea[10], and counterrolling of eyes and perception of vertical in humans[11-15]. These reactions are influenced by both parameters which determine shear, that is to say, they depend as much on the direction (which can be changed by tilting the subject) as on the magnitude of the gravito-inertial force.

In the octopus statocyst, the oval shaped sensory epithelium (macula) of the gravity receptor is oriented vertically in the normal position of the animal[16]; the macula planes of the left and right statocyst subtend an angle of 90 degrees open to the front. The sensory elements of the macula show a clear morphological polarization: the basal feet of all kinocilia of one cell project radially away from the centre of the macula[17-19].

We investigated the counterrolling of the eyes of *Octopus vulgaris*. This compensatory reaction depends on the function of the statocysts[20]. It was recorded in seventy-two specimens at normal gravity (experiment *A*) and in seven specimens at increased magnitude of gravito-inertial force in a centrifuge (experiment *B*). The animal was mounted with its arms firmly fixed together on a board which could be rotated in the sagittal plane (except in experiment *A*(4)), that is, around a transverse axis of the animal, using the eye as a pivot point. Rotation was carried out with a motor with constant speed of 2·4 degrees/s. The position of the pupil was recorded photographically every 20 degrees. In Fig. 1, the deviation of the pupil slit from an animal fixed reference line is plotted against the animal's position to gravity. *A*(1) The two curves of each diagram refer to forward and backward rotation of the animals. Those of Fig. 1(1), (2) and (4) demonstrate a hysteresis effect (B.-U. B., in preparation). *A*(2) In thirty-eight specimens the function of one gravity receptor was abolished by carefully displacing the statolith completely from the macula. The curves are of the same shape as those of normal animals, but the amplitude is diminished by about 50 per cent (compare Fig. 1(1) with Fig. 1(2)). No difference could be observed either in the movements of the left and right eye or in the eye movements of left and right operated animals (B.-U. B., in preparation). Unilateral extirpation of the whole statocyst sac resulted in similar curves, but a small difference in the amount of counterrolling of both eyes was observed during the first days after operation (B.-U. B., in preparation). *A*(3) After bilateral extirpation of the whole statocyst sac and after bilateral displacement of the statoliths no counterrolling of the eyes was observed (B.-U. B., in preparation). *A*(4) In twelve unilaterally operated animals, the counterrolling was recorded in the following positions of the remaining macula plane relative to the rotation axis: (*a*) 45 degrees, that is, rotation around the transverse axis of the animal as in experiment *A*(1) (Fig. 1(2)); (*b*) parallel (Fig. 1(3)); or (*c*) perpendicular (Fig. 1(4)). The animals with 45 degree and perpendicular oriented macula showed almost normal counterrolling. In the animals with parallel oriented macula, however, the counterrolling had ceased almost completely.

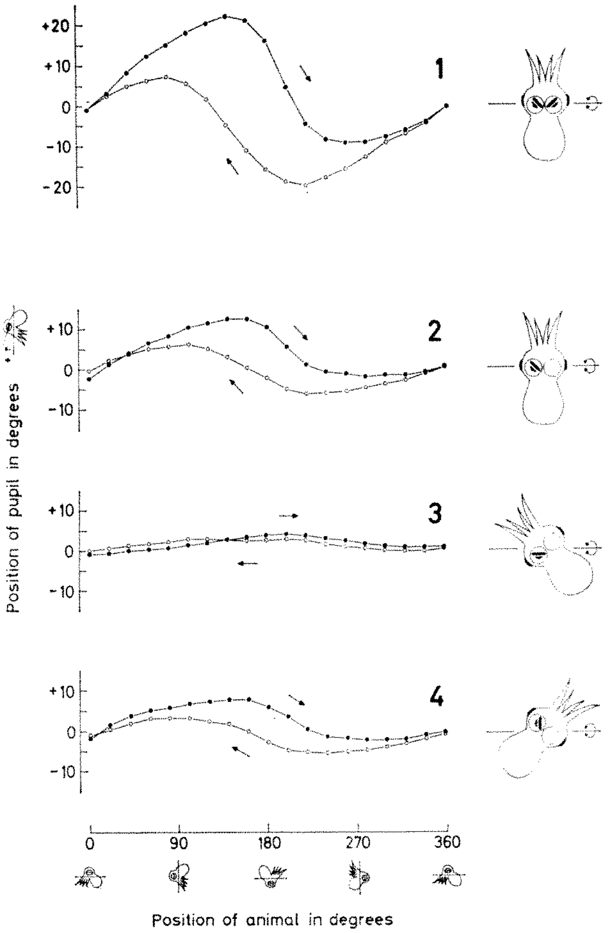

Fig. 1. Compensatory counterrolling of the eyes of octopus as a function of body position to gravity. Each curve represents the averaged data of twenty-four records on the same twelve animals. Ordinate: angle between pupil slit and animal fixed reference line (which is horizontal in the normal position of an octopus); abscissa: angle of tilt from the normal position. Tilt was performed around rotation axes as indicated in the symbols on the right hand side. Each symbol shows an octopus seen from above, with statocyst(s), macula(e) and statolith(s). Arrows on the curves refer to direction of tilt. The four diagrams refer to the results from: 1, normal animals; 2, unilaterally operated animals, rotation axis in a 45 degree position to the macula plane, that is, rotation axis transversal to the animal (as in 1); 3, unilaterally operated animals, rotation axis parallel to the macula plane; 4, unilaterally operated animals, rotation axis perpendicular to the macula plane.

B. Normal and unilaterally operated animals were investigated in a centrifuge at different body positions at magnitudes of gravito-inertial force from 1·0*g* to 1·5*g* (in our experiments centrifugation affected only the magnitude, not the direction of the force with respect to the animal). As can be seen from Fig. 2, the magnitude of the gravito-inertial force did not influence the counterrolling of the eyes.

These results lead to the following conclusions. (1) The counterrolling of the eyes of octopus depends on the function of the statolith organ of the statocyst sac (experiment $A(3)$). It does not seem to be affected by sensory elements of the cristae which are known to be stimulated by angular acceleration[21]. This view is suggested by the absence of angular acceleration in our experiments and also by the results of experiment $A(4)$ (Fig. 1(3)). (2) The effect of both gravity receptors on the counterrolling is additive (compare Fig. 1(1) and Fig. 1(2)). Similar results have been obtained in fish[9] and Crustacea[10]. (3) The ocular counterrolling alters as a function of the direction of shear of the statolith (Fig. 1(4)). (4) The counterrolling is not affected, however, by the magnitude of shear which has been altered either by change of tilt position of the macula (Fig. 1(3)), or by change of magnitude of gravito-inertial force (experiment B).

Although the eye counterrolling in octopus is not affected by the amount of shear, shear (lateral displacement) may nevertheless be considered as the stimulating force for the sensory elements of the macula. The topographical arrangement of the maculae implies that with change of position of the animal, for instance, around a traverse axis, the direction of the shear force of the statolith moves on the macula like a hand on a clock-face. The current theory of stimulation of sensory cells bearing kinocilia[6-8,18,19] assumes that the excitation of a sensory cell increases when the kinocilium is displaced towards the basal foot and decreases when displaced in opposite direction; the excitation varies as a function of the cosine of the angle of projection of the basal foot relative to the direction of the stimulus, that is, of statolith shear. In octopus this would result in a specific pattern of excitations of all sense cells on the macula. It can be characterized by a maximum of excitation of the cells which are oriented with their basal feet in the direction of statolith shear. From the direction of maximum stimulation, the excitation of the cells in both directions decreases as a function of the cosine of the angle of the basal foot orientation to the direction of statolith shear.

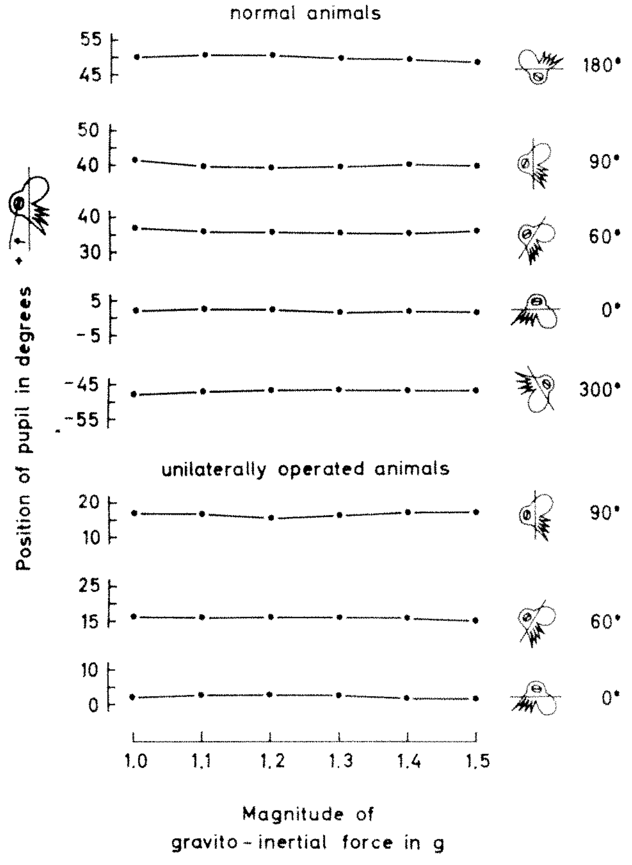

Fig. 2. Compensatory counterrolling of the eyes of octopus as a function of the magnitude of the gravito-inertial force at the body positions indicated on the right hand side. Each curve consists of single measures on one animal. Ordinate: angle between pupil slit and animal fixed reference line; abscissa: magnitude of gravito-inertial force.

As can be concluded from our data, only the angular orientation of the excitation pattern on the macula, that is, its rotatory shift with respect to macula fixed coordinates, affects the counterrolling of the eyes of octopus. This is irrespective of the overall amount of excitation as affected, for instance, by shear magnitude. Thus these findings reveal a principle of statocyst function which seems to be different from that described for Crustacea and vertebrates.

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Eye Ultrastructure, Colour Reception and Behaviour

IN many animals—both vertebrates and invertebrates—certain innate behavioural patterns (in, for example, feeding and sexual behaviour) are preferentially elicited by stimuli with specific qualities (such as colour of visual stimuli) which act as so-called releasers. An initial "tuning" of a stimulus as a releaser may take place at the receptor level (as with receptor specificity in night moth olfaction). One might ask whether, in some cases, further mechanisms may exist in the transmission of energy to the receptors which enhance selectively that quality of the stimulus which characterizes it as a releaser.

The present study shows that, in a diurnal butterfly (*Heliconius erato*), such a mechanism in the transmission system to the photoreceptors selectively enhances the stimulating effect of light within a certain wavelength region, of which the hue represents the releasing factor for a well described innate behaviour pattern.

The compound eyes of many invertebrates (such as the crayfish and butterfly), as well as the camera eyes of several other invertebrates (such as the scallop) and certain vertebrates (such as dog, cat and sheep), are equipped with an array of various types of specialized structure behind the photoreceptors. The array acts as a mirror, reflecting light back to the receptors, and thus increases the chance of capturing extra quanta. When the compound eye of a dark-adapted night moth is illuminated, such a reflexion ("glow") can be seen in a great many ommatidia in the part of the eye facing the

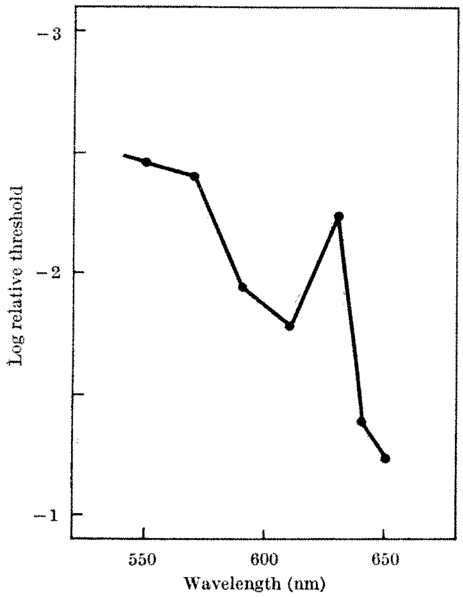

Fig. 1. Log relative threshold of electrical response from compound eye of *Heliconius erato* to monochromatic light between 550 and 650 nm (duration of stimulus 0·2 s).

observer[1]. This non-selective reflexion in night moths comes from the tracheolar layer around and behind the proximal ends of the receptors[2]. In diurnal butterflies a discrete coloured glow can be seen with the aid of the ophthalmoscope, which makes possible observation and illumination from the same direction[2]. In recent years, attention has been paid to various microcomponents of the compound eye optic system which may influence the energy transmission to the receptors[3-6]. It has been shown[5-6] that the selective reflexion, expressed in the coloured glow in the diurnal butterfly, occurs in a multi-layered structure of platelets formed by modified taenidia of the tracheoles located behind the receptors. This structure functions as a quarter-wavelength interference filter, reflecting light in its rejection band. It was suggested[5] that these structures, which seem to be characteristic for diurnal butterflies, serve to increase colour contrast.

The behaviour of the diurnal tropical butterfly, *Heliconius erato* has been thoroughly studied by Crane[7], who found that in this species red light—of a colour similar to that of the butterfly's forewing band—is an important releaser in courting behaviour. In our study, therefore, this species was used for ophthalmoscopic, electron microscopic and electrophysiological investigation.

Ophthalmoscopy with white light revealed a red glow, representing selective reflexion of light of the forewing band colour. Comparison with monochromatic light, made by independent observers, showed that the colours of both the glow and the forewing band were in the wavelength range 600–650 nm. Electron microscopy showed the existence of numerous wide, straight tracheoles around and behind the photoreceptors. Calculations based on the dimensions of the taenidial array of these strongly developed tracheoles indicate that this tracheolar system could serve as an interference filter (compare Miller *et al.*[5]) reflecting the red light seen in the glow.

To see whether this reflexion of light in the red band enhances selectively the stimulating effect in the red wavelength region, measurements were made of the intensity of monochromatic light required to elicit a threshold compound photoreceptor response of 50 µV. The eye was stimulated with light of different wavelengths (380–650 nm) by using a prism monochromator (Zeiss *M* 4 *Q* III) and the electrical response was recorded with Ag–AgCl electrodes (diameter of corneal electrode 0·1 mm).

The general shape of the spectral sensitivity curve, which is of less interest in this context, was similar to that described for other insects (for a review, see Burkhardt[8]). Accordingly, the sensitivity decreased between 550 and 650 nm, as seen in Fig. 1. On the slope of the curve towards longer wavelengths, however, there is a pronounced and narrow peak (about 0·5 log units) between 610 and 640 nm. The narrowness of the peak, together with the fact that such a peak has never been obtained in Lepidoptera lacking selective reflexion (such as night moths[9]), make it unlikely that the peak represents a specific photopigment. Because it is localized within a wavelength region corresponding to that of the observed glow, the peak seems rather to result from selective reflexion in the red band caused by the tracheolar system. The peak represents a significant enhancement of the stimulative efficiency.

Our results thus indicate that the reception of a colour cue serving as releaser of an innate behavioural pattern can be selectively enhanced by a specialized mechanism of the visual dioptric system.

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Arousal and Information Selection in Short-term Memory

In experiments on short-term memory a subject is typically presented with a list of items such as words or digits and then asked to recall the complete list in order of presentation. This simple paradigm has produced many important findings about the nature of the information-handling mechanism in memory. In most cases, however, the problem of short-term memory is assumed only to involve storage and retrieval of information, and no allowance is made for the subject's freedom to attend selectively to the incoming stream of information. In other words, some of the information presented may never be admitted to storage at all—a possibility that is particularly relevant to recent research[1-4] on the interaction between memory processes and arousal, because we have found[5,6] that arousal is a major determinant of changes in patterns of information selection in visual monitoring tasks. In loud noise, subjects attend more to sources of information of "high priority" and are more likely to disregard sources of less important information.

The findings mentioned above show an interesting relation between arousal, or general level of stimulation, and the length of delay between the presentation of items and their recall. Higher arousal in these studies has been either inferred from larger autonomic responses to particular stimulus words, or produced by carrying out the task in a stimulating environment (in practice, white noise has been used for this purpose). In general, high arousal items are recalled less well immediately after presentation than those associated with low arousal, but are produced more readily in a recall test which is delayed by, say, 24 h. The only present theory to account for these results is based on the concept of "trace consolidation", and has been advanced particularly by Walker and Tarte[2]. This view argues that the presence of a raised state of arousal at the time an item is presented results in a "more active" memory trace, so that when the consolidation process is completed (perhaps the next day) recall is better for these items. To account for the impairment in immediate recall of high arousal items, increased consolidation of the trace is postulated to be incompatible with its retrieval, because the two processes can be regarded as opposite in direction. The effect, that is, relates only to differential consolidation of the input once it has been taken in. In view of our own findings, that the pattern of information selection seems to change with arousal, at least part of the effect may conceivably be the result of differential intake of information.

We are now examining this possibility directly in another series of experiments. The present study, however, deals only with demonstrating changes in input selection in the memory situation with arousal (noise): the simple short-term memory paradigm outlined at the beginning is used. If information selection changes occur in memory just as they occur in monitoring situations when arousal is increased, then we should find that subjects attend relatively less to an irrelevant source of information in noise. To test this, an extra feature is incorporated into the short-term memory situation—the presence of an irrelevant cue. The relative performance of subjects on the relevant and irrelevant parts of the task can then be used to infer any changes in attention distribution under arousal.

Eight slides were made up, each containing a common bisyllabic adjective. The irrelevant cue was that each word could appear in any of the four corners of the screen (two words, in fact, appeared in each corner). The words were presented at a fixed rate of one every two seconds, no two successive slides presenting words in the same location. Subjects were told nothing about the location cue; they were instructed to recall all the words, in correct order, when they observed the blank screen at the end of the series. A sheet was provided with eight spaces in which to write correct responses. After all subjects had completed the relevant task, it was pointed out that the words had, in fact, appeared in different locations, and they were asked to indicate these by putting a cross in the corresponding corner for each of the words they had written down. They were told to guess if they could not remember.

Sixty-eight subjects were tested in groups in one of two experimental conditions: (*a*) "quiet"—a continuous background noise of about 55 dB was present in the testing room, and (*b*) "noise"—the level was 80 dB. Thirty-six subjects were tested in the quiet condition and thirty-two in noise. The noise level remained constant throughout the experiment for each group of subjects. Instructions were given with no noise present.

Three performance measures were taken, as shown in Table 1. For the relevant task, recall was scored by considering (i) both word and order accuracy, and (ii) word accuracy only, ignoring the necessity for words to be in the correct order. The second measure tended, naturally, to give larger scores. Neither measure shows any difference between noise and quiet (both $P > 0{\cdot}05$). There is a tendency, however, for recall in noise to be better when correct order is taken into consideration (this comparison almost reaches statistical significance at $P = 0{\cdot}05$). The difference disappears completely and tends to reverse when order is not considered, implying that subjects in the noise condition attend more to order cues present in the list (links between successive words or direct coding of order for each word). Success on the irrelevant task (recall of locations) was best measured by taking the mean percentage of correct locations as a function of the total number of words correctly recalled (measure (ii) above). This was necessary because the possible number of correct locations is limited by the number of words successfully reported (a subject cannot attempt to recall the location of a word he has not recalled). This measure shows a significant difference between treatments ($P < 0{\cdot}05$). Attention to the irrelevant cue thus occurs less in the noise condition, subjects selecting information more from the relevant task (this is supported by the above observation about order preservation in the recall of the word lists).

Table 1. MEAN PER CENT RECALL OF RELEVANT AND IRRELEVANT CUES IN "QUIET" AND "NOISE"

	Relevant task Percentage of words recalled in correct order	Percentage of words recalled irrespective of order	Irrelevant task Percentage of locations recalled
Quiet ($N = 36$)	43·75	73·12	48·50
Noise ($N = 32$)	54·12	69·00	32·00
Statistical significance	$P = 0{\cdot}055$	$P > 0{\cdot}10$	$P < 0{\cdot}05$

These findings illustrate that changes in the pattern of information selection are a measurable feature of memory situations. It cannot be assumed that all relevant information is taken in equally efficiently in different conditions. There is a tendency for subjects, even in this simple and brief task, to attend to features of the stimuli which are not relevant to efficient performance (as defined by the instructions). This tendency occurs less in situations that are arousing, and there may thus be important consequences for the general applicability of the consolidation theory to the findings on arousal and memory discussed earlier. In particular, it seems likely that at least part of the explanation for these results will involve differences in information intake with high and low arousal. It is also worth noting that immediate recall with high arousal tended to be better in this experiment when order preservation was a criterion for success, a result that cannot easily be accounted for by the consolidation theory discussed earlier. We are now attempting experimentally to clarify further the roles of attention and consolidation in memory. A preliminary interpretation is that, with high arousal, subjects take in less information but pay more attention to it. This could give a variety of results for the short-term, depending on the ease of the task, readiness to guess, and so on, but in the long-term should always give better recall for high arousal because the consolidation process has a larger or more well-defined trace on which to operate.

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Antagonistic Effects of Tryptamine and β-Phenylethylamine on the Behaviour of Rodents

ACCORDING to Brodie and Shore, the behaviour of higher animals depends on neurohumoral agents produced in the diencephalon. Catecholamines such as noradrenaline and dopamine are said to act as ergotropic agents, whereas serotonin is their antagonistic trophotropic factor[1,2]. The essential validity of these concepts has been confirmed many times[3,4]. There are, however, some discrepancies concerning the supposed stimulating (ergotropic) effects of catecholamines; some results confirm their alerting action, but others point to a depressing effect[5,6]. We found[7–11] that not only catecholamines, but also their amino-acid precursor L-dopa, suppress exploratory behaviour in mice, whereas amphetamine derivatives, as well as β-phenylethylamine and its amino-acid precursor phenylalanine, enhance it; there is a mutual antagonism between the two groups of substances. Serotonin too suppresses exploratory activity in mice and produces a Parkinson-like state in rats, similar to that produced by reserpine. β-Phenylethylamine antagonizes these effects of serotonin[12]. We concluded that β-phenylethylamine rather than catecholamines should be considered the ergotropic agent, while serotonin, as proposed by Brodie and Shore, is its antagonistic trophotropic factor. It seems to us that catecholamines may function differently in particular systems, but they do not seem to stimulate motor activity. Amphetamine and its derivatives may act by liberating β-phenylethylamine.

Table 1. EFFECT OF β-PHENYLETHYLAMINE DERIVATIVES ON RESERPINE AND TRYPTAMINE PARKINSON-LIKE SYMPTOMS IN THE RAT

Drug	Dose (mg/kg)	No. of animals	Animals with Parkinson-like symptoms Catatonia	Akinesia	Tremor	Rigidity
Reserpine	5	20	18 (90%)	19 (95%)	19 (95%)	18 (90%)
Reserpine + β-phenylethylamine	5 50	20	1* (5%)	1* (5%)	20 (100%)	2* (10%)
Reserpine + methylamphetamine	5 2	20	1* (5%)	1* (5%)	20 (100%)	1* (5%)
Tryptamine	80	20	10 (50%)	19 (95%)	19 (95%)	0
Tryptamine + β-phenylethylamine	80 50	20	2* (10%)	2* (10%)	20 (100%)	0
Tryptamine + methylamphetamine	80 2	20	1* (5%)	1* (5%)	20 (100%)	0

* Values statistically significant ($P < 0{\cdot}01 > 0{\cdot}001$).

Dewhurst reached similar conclusions[13–15], except that he included tryptamine among the stimulating agents, maintaining that phenolic amines such as catecholamines and serotonin are depressing, whereas non-phenolic amines such as β-phenylethylamine and tryptamine are stimulating. Nevertheless, according to our results, tryptamine affects the behaviour of mice and rats in a manner very similar to that of serotonin. β-Phenylethylamine is an antagonizing rather than a synergizing agent to the effects of tryptamine. We have measured effects on exploratory activity in mice, using an actophotometer[16], and we have produced a Parkinson-like state in rats. Twenty female hooded rats (180–280 g body weight) received each of the doses indicated in Table 1. Drugs were dissolved in distilled water and injected intraperitoneally, 1 ml./100 g body weight. β-Phenylethylamine derivatives were administered 2 h after reserpine and 30 min after tryptamine. Results were evaluated by an "all or none" reaction as follows. Catatonia was considered to be present when the rats, put on a table with their forelimbs placed on a platform 6 cm high, remained still for at least 1 min. Akinesia was found when the rats made no exploratory movements for 2 min at least. Rigidity was present when the rats allowed themselves to be dragged by the tail without making escape movements. Absence or presence of tremor was assessed visually. Because tryptamine does not produce rigidity, this was not tested in assays with this substance.

The degree of phenylethylamine antagonism was expressed as the percentage of rats which still had Parkinson-like symptoms. Statistical significance was assayed according to Bancroft[17].

In our other investigations groups of six male white mice (18–30 g) were put in individual cages, and injected intraperitoneally 30 and 15 min before locomotor activity was measured for 10 min in the actophotometer. Reserpine was always administered 24 h before test. Results are shown in Table 2.

Table 2. EFFECTS OF RESERPINE, TRYPTAMINE, AND β-PHENYLETHYLAMINE DERIVATIVES ON THE EXPLORATORY BEHAVIOUR OF MICE

First injection	(mg/kg)	Second injection	(mg/kg)	Actophotometer reading (mean ± *S.E.*)
Distilled water		Distilled water		65 ± 5
Tryptamine	80	Distilled water		8 ± 4*
Reserpine	2	Distilled water		7 ± 2*
Distilled water		Methylamphetamine	2	101 ± 8*
Distilled water		β-Phenylethylamine	80	90 ± 9
Tryptamine	80	Methylamphetamine	2	101 ± 14*
Tryptamine	80	β-Phenylethylamine	80	91 ± 14*
Reserpine	2	Methylamphetamine	2	174 ± 14*
Reserpine	2	β-Phenylethylamine	80	48 ± 8*

* Results statistically significant ($P < 0{\cdot}01 > 0{\cdot}001$).

Tables 1 and 2 show that reserpine as well as tryptamine produces hypokinesia in mice. The reserpine syndrome in rats consists of catatonia, akinesia, tremor and rigidity, whereas tryptamine only produces catatonia, akinesia and slight tremor. These symptons, with the exception of tremor, which is enhanced, are antagonized by β-phenylethylamine and methylamphetamine. It seems that tryptamine produces not stimulation, but depression-like phenomena, and that β-phenylethylamine acts as its antagonist. Tryptamine as well as β-phenylethylamine passes the blood–brain barrier[13,18] and so there can be no objection on this count. We cannot explain the differences between our results and those of Dewhurst; maybe they have resulted because we used different animal species (rodents rather than newly hatched chickens) or because of some other experimental details.

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Effects of Sexual Activity on Beard Growth in Man

DURING the past two years I have had to spend periods of several weeks on a remote island in comparative isolation. In these conditions I noticed that my beard growth diminished, but the day before I was due to leave the island it increased again, to reach unusually high rates during the first day or two on the mainland. Intrigued by these initial observations, I have carried out a more detailed study and have come to the conclusion that the stimulus for increased beard growth is related to the resumption of sexual activity.

Beard growth was measured quantitatively by collecting and weighing the shavings from the head of a Philips Philishave razor after a single shave once every 24 h. Activity data were recorded on a 0–5 scale with respect to physical and mental exercise, nervousness, sleep, libido and intercourse.

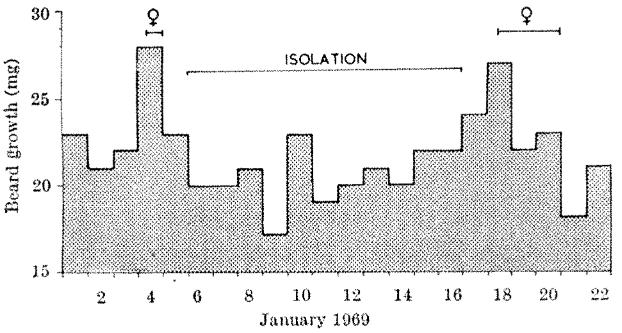

Fig. 1.

Fig. 1 illustrates the changes in beard growth during a short stay on the island. The day of return to the mainland and the initial resumption of sexual activity produced a most marked increase in beard growth, although growth subsequently declined fairly rapidly to baseline rates. If sexual activity was maintained for a week or more, then beard growth would still decline to normal rates within 4–6 days; resumption of sexual activity after only 2–3 days' abstinence was enough to restimulate beard growth. Even the presence of particular female company in the absence of intercourse, after a period of separation, usually caused an obvious increase in beard growth.

Perhaps the most interesting feature of these changes was the way the increase in beard growth anticipated the resumption of sexual relationships. The longer the period of abstinence, the more obvious was the anticipatory response. Fig. 2 shows the mean beard growth (± *S.D.*) for ten different 10 day recording periods in which sexual relationships were confined to the weekend. The cycle is unmistakable; the Friday peak comprises an anticipatory response during the day and the effect of intercourse; the increased beard growth falls off by Sunday, and by Monday it becomes smaller than on any other day of the week.

Although normal sexual relationships seem to have the most pronounced effects on beard growth, other factors are also of importance. Increasing the number of shaves in a day leads to a considerable apparent increase in beard growth, although decreasing the frequency of shaving did not necessarily have the opposite effect. Tension, anxiety, nervousness, excessive mental fatigue and alcohol consumption were all associated with increased beard growth, while excessive physical exercise and high ambient temperatures seemed to inhibit growth. The amount of sleep, type of food eaten and degree of libido were without effect.

Hamilton[1], in a comprehensive account of the effects of genetics, age and hormone levels on beard growth in man, stated that beard growth could be used as a bioassay for androgens because it was exclusively under testicular control. An attempt was therefore made to correlate the rate of beard growth with a known index of androgenic activity, the rate of sebum production by the sebaceous glands of facial skin[2]. The results are shown in Fig. 3; in general, there was good agreement between these two indices of androgenic activity.

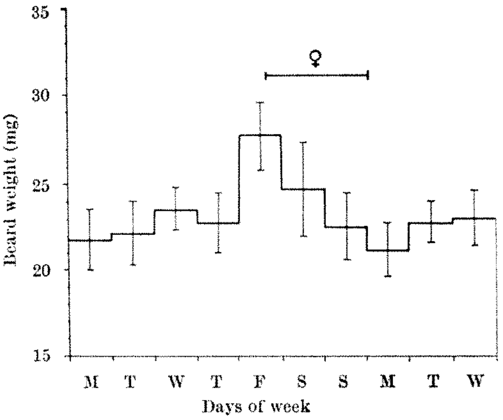

Fig. 2.

A variety of compounds, including testosterone, methyl testosterone, androsterone and cortisone (2 × 10 mg/day), were taken sub-lingually and beard growth changes measured. All the samples were coded and placebos were also included. The results show that all the androgens had a stimulatory effect on beard growth, androsterone producing the greatest increase. The changes were of the same order of magnitude as the increases associated with intercourse. Although the placebos were without effect, cortisone also stimulated beard growth, indicating that the beard may respond to other hormones.

In conclusion, it seems that beard growth in man is a much-neglected parameter of hormone activity that can readily be quantified. This study would suggest that the changes in beard growth in relation to everyday activities may reflect changes in the endocrine activity of the gonads or adrenal cortex. The fact that the beard shows a marked circadian rhythm of growth with a peak between 0800 and 1300 h, similar to the circadian rhythm of cortisol secretion, might suggest a functional interrelationship. Because the beard is a secondary sexual character developed under the influence of testosterone at puberty, however, it might be expected to retain a particular sensitivity to androgens. The pronounced effect of sexual intercourse on beard growth may therefore be caused by an increase in testosterone secretion, which is known to

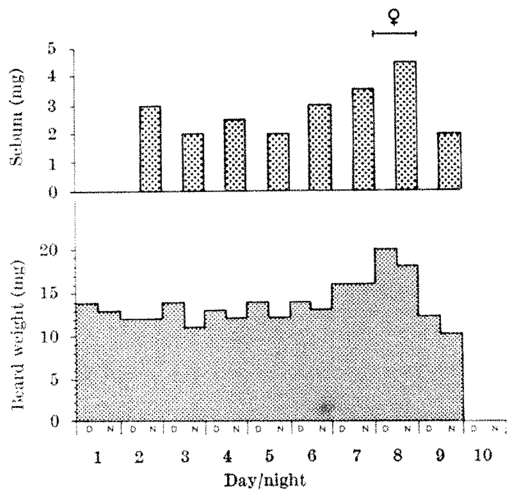

Fig. 3.

occur in response to coitus[3]; there is a 5–8 day cycle of testosterone secretion, and a rather similar 5–6 day cycle is sometimes apparent in beard growth.

Changes in beard growth may well indicate changes in androgen secretion in man, related to sexual behaviour, in that they suggest a release of androgen in anticipation of intercourse, a decline in androgen secretion with continued sexual activity, and hypersecretion of androgen in response to intercourse after prolonged sexual abstinence.

ANON

The identity of the author of this communication has been suppressed for reasons which may be self-evident, but the author, whose work has been vouched for by a colleague, has answered a number of questions raised by a referee.

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Efficiency of Grass Carp (*Ctenopharyngodon idella* Val.) in controlling Submerged Water Weeds

THE Asiatic grass carp is a herbivorous fish which is being bred and extensively cultured for food in Russia and eastern Europe. In Britain where freshwater fish are seldom eaten, apart from eels and salmonids, grass carp are arousing interest because of the possibility of using them as a supplement, or in some situations as an alternative, to present methods of weed control—mechanical cutting or the use of herbicides[1]. The chief factors involved in the possible use of grass carp have been discussed[2,3], one of the most important being the density of fish needed to achieve a desired degree of control. We report here the work done on this topic in 1969.

Nine similar and adjacent ponds, each 0·025 hectares in size and about 0·8 m deep, were cleared of indigenous fish by using rotenone. The rather heterogeneous weed growth present was sampled by means of a grapnel at regular intervals along evenly spaced transects and recorded as relative frequencies, that is, the number of samples in which submerged weeds appeared as a percentage of the total number of samples per pond. Between forty-five and fifty-five samples were taken at each pond.

A stock of grass carp had been obtained in April 1968 from Hungary, where they were hatched in June 1967. After the first weed sampling the fish were introduced into six of the nine ponds on May 21 at the rates of 238, 241, 242, 484, 716 and 959 kg/hectare. The mean weight of individual fish was 168 g. Subsequent assessments of weed abundance were made in July, August and September.

In initial approximate order of abundance the submerged plants present were: *Myriophyllum verticillatum*, *Callitriche* sp., *Lemna trisulca*, *Potamogeton pectinatus*, *Zannichellia palustris* and *Ceratophyllum demersum*. The emergent and chiefly peripheral species were *Typha latifolia*, *Sparganium erectum* and *Alisma plantago-aquatica*. Although not all these species were present in each pond, when they occurred in ponds stocked with grass carp they were all grazed.

During the summer all species of weed in the ponds containing the fish decreased while the weed density in the three control ponds increased to a mean value of 110 per cent of the original. In the most heavily stocked pond the relative frequency of submerged weeds decreased from eighty-four in May to eighteen in August when plant roots—presumably pulled up by the fish—were seen floating on the water; it was clear too that the lowermost leaves of *T. latifolia* had been eaten. By September all traces of submerged plants had gone and all the *T. latifolia* leaves touching the water had been attacked by the fish. Grass carp are known to prefer some plants to others[4–7], and our results suggest that *Callitriche* sp. was preferred to *M. verticillatum*, but the initial variation in plant population between ponds and the method of assessment did not permit a reliable comparison.

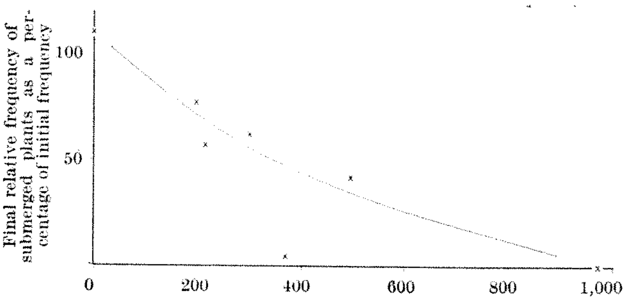

Fig. 1. Relation between the change in the relative frequency of submerged weeds from May to September and the grass carp stocking rate.

On September 22 and 23 the grass carp populations were estimated by a mark-recapture method and the fish were subsequently removed, weighed and measured. Mean survival was 61 per cent with a range of 34 to 97 per cent and the mean weight increment was 37·8 g (7·7–139 g).

From the initial stocking figures and the final numbers and weights of fish, a median-time biomass was estimated for each pond, assuming growth and mortality to have been linear and exponential respectively. The values obtained are plotted in Fig. 1 against the September relative frequencies of submerged weed expressed as a percentage of the May values and give a reasonably good curvilinear relation. The very low point in Fig. 1 is believed to be due to the fact that the initial plant population was low in this pond and consisted chiefly of *Callitriche* sp., which is not only readily eaten by grass carp but also tends to die back as the summer progresses as it did in the control ponds.

Thus in the summer of 1969 at a site where the daily mean water temperature was 15·8° with a range of 8·5° to 21·5° C, and with plants which are readily eaten, a stocking density giving a mid-season biomass of approximately 300 kg of 2 year old fish per hectare reduced plant growth to about 50 per cent of its potential. In the conditions of the experiment this mid-season biomass would, it is estimated, have required an initial stocking rate of 343 kg/hectare.

We thank the Deeping Fen, Spalding and Pinchbeck Internal Drainage Board and the Welland and Nene river authority for facilities for this work.

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Absolute Pollen Diagram from the British Late-Glacial

BLELHAM BOG, a National Nature Reserve 1·2 km west of Windermere, overlies two small infilled kettleholes in gravels of the latest major glaciation. In the western kettlehole the succession of late-glacial sediments includes two organic layers (carbon content approximately 20 per cent of dry weight) respectively 5 cm and 40 cm thick, separated by a silt of low organic content (carbon about 6 per cent). Two radiocarbon dates from the lower organic layer, 12,380 BC ± 230 and 12,330 BC ± 230 (Q 758), and descriptions of stratigraphy, pollen analysis, diatoms and Cladoceran remains in the late-glacial sediments have been published[1-5].

A grant from the Royal Society to W. P. made it possible to obtain ten further dates from the profile, from the firm Isotopes, and so to determine the changes in annual pollen deposition per unit surface area of lake mud. Using the time scale based on the eleven closely spaced radiocarbon dates (Fig. 1) and pollen counts related to a measured volume of fresh sediment, it has been possible to prepare an absolute pollen diagram in the manner of Davis and Deevey at Rogers Lake[6]. The pollen counts per unit volume were carried out by a modification of the technique outlined by Benninghof[7] and described by Matthews[8] using an added suspension of exotic pollen grains. A full description of this modified technique will be published by A. P. B. with details of the statistical methods by which the confidence limits of the counts were calculated.

The ten additional dates confirm the very early date of the lower organic layer (Q 758) and provide a consistent pattern of three successive Late-Weichselian periods, each differentiated stratigraphically and characterized by a different accumulation rate. We recognize that the dates, like all radiocarbon dates from lake mud, are open to criticism because of possible incorporation of ^{14}C deficient carbon during the photosynthesis of aquatic plants, even though these sediments are non-calcareous. Fig. 1 shows how the time scale based on these dates assigns a length of about 500 years to the post-Alleröd period of severe soil erosion which produced minerogenic sediment at Blelham Bog; this agrees closely with the generally accepted length of this period, Younger Dryas[9]. The dates for the beginning and end of this period on the Blelham Bog time scale are between 300 and 500 years older than those usually given in recent reviews[9,10].

Fig. 2*a* shows the pollen zones into which the percentage pollen diagram can be divided, and the correlation of these with lithostratigraphic units. Fig. 2*b*, the absolute pollen diagram, is divided by the zone boundaries which are defined on the percentage pollen diagram.

Conclusions from study of the absolute pollen diagram must be: (i) Zone B*a*, defined by predominance of *Rumex* (mainly *acetosa* type) and grass pollen, represents a section of the profile of very low pollen deposition per year throughout. On the uncorrected time scale this section represents the period from just before 13,000 BC until about 11,000 BC. Within this section the lower organic layer must represent only a local environmental fluctuation within the former kettlehole pond, for it is not differentiated from the minerogenic deposits above and below it by any significant change in pollen input. The percentage increases in pollen of juniper and tree birch within the organic layer[1,4] "represent statistical artefacts rather than significant vegetational changes"[6]. The low values per unit area per year for tree birch and juniper grains indicate that these high pollen producers cannot have been contributing materially to the local pollen grain, so grains of these taxa in deposits of Zone B*a* must have been derived chiefly from distant transport.

(ii) Zone B*b*'s lower boundary corresponds to an increase, more than ten-fold, in the total input of pollen grains per year per unit area. The input of all taxa increases, but the rapidity of the absolute increase in juniper produces the high juniper percentages which define zone B*b*. Both the herbaceous taxa characteristic of Zone B*a* and tree birch pollen increase absolutely at the zone boundary B*a*/B*b*; the annual input of *Betula* grains increases through the deposits of Zone B*b* to reach maximum values in Zone B*c* above. At the zone boundary B*b*/B*c* a steep fall in the annual input of juniper begins. Input of the herbs, grasses, sedges, *Rumex* and *Thalictrum* increases by a mean factor of about 6 at the zone boundary B*a*/B*b*, and remains at about this level through Zones B*b* and B*c*. *Artemisia* has consistently higher absolute values in Zone B*b* than in B*a* or B*c*.

These facts can most easily be explained by the hypothesis that the zone boundary B*a*/B*b* represents a major change in pollen input, most probably the result of a rise in temperature. Herbaceous plants of all communities represented by the pollen spectra of Zone B*a*, on this theory, responded by increased flowering and pollen production. Juniper, hitherto stunted and sparsely flowering, responded immediately by the production of erect flowering branches, as suggested by Iversen[11]. The slow increase in pollen input from tree birches, beginning at the zone boundary B*a*/B*b*, implies a slower response to environmental change, explicable by the time-lag involved in migration of tree birches from distant sites. When birch trees reached the site, juniper would be shaded out, producing the steep fall in pollen input of juniper at the B*b*/B*c* boundary. Sustained input from grasses, sedges and *Rumex* through Zones B*b* and B*c* indicates that the spread of the woody plants did not materially reduce pollen input from herbaceous communities.

(iii) Zone B*c*, the *Betula* zone, represents ecological equilibrium during the full development of the interstadial. Within the next zone, B*d*, pollen input of *Betula* falls irregularly from its maximum in B*c*, and that of *Juniperus* increases. Irregular break-up of birchwoods with increased flowering of juniper would produce these results. The "forked" percentage curve for *Betula* resembles that found at many Danish sites[12] and used there to divide Zone II into *a*, *b* and *c*. A similarly forked

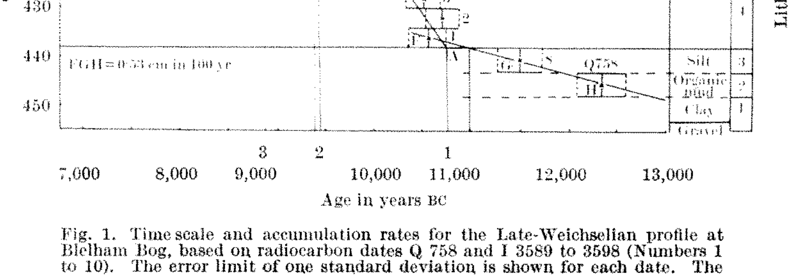

Fig. 1. Time scale and accumulation rates for the Late-Weichselian profile at Blelham Bog, based on radiocarbon dates Q 758 and I 3589 to 3598 (Numbers 1 to 10). The error limit of one standard deviation is shown for each date. The graph showing accumulation rate has been plotted as follows: DE and FGH represent sections where three dates fall on a straight line, and ABC represents the line of best fit calculated for samples 1 to 7 inclusive. The numbers 1, 2 and 3 on the lower axis show the positions on the time scale of the three major changes in lithostratigraphy.

curve for input of *Filipendula* pollen at Blelham Bog agrees with the interpretation of this as the result of a fluctuation in temperature.

(iv) At the zone boundary B*d*/B*e* total annual input and numbers of grains of *Betula* and *Juniperus* fall steeply, *Juniperus* to an absolute minimum for the profile. Increased input, first of *Rumex* (mainly *acetosa* type) and then of *Artemisia*, produces the successive maxima in the percentage diagram on which pollen zones B*e* and B*f* are defined. Pollen input is low, but higher than in the pre-interstadial period. The increased input of *Artemisia* represents a higher contribution from plants of disturbed soils than at any other time during the Late-Weichselian.

(v) Pollen zones B*g* and B*h* represent the transition to the basal *Betula* zone of the Flandrian.

Here is evidence for a single interstadial in a Late-Weichselian profile for which the radiocarbon dates go back to 12,380 BC ± 230. Pollen zone B*a* corresponds, in the predominance of non-woody taxa, to Zone I; pollen zones B*c* and B*d* together equate with the classical Alleröd or Zone II, in which tree birches dominate the pollen spectra; and pollen zones B*e* and B*f* together equate with the post-Alleröd recession, Zone III. The peculiar feature of the Blelham Bog profile is the evidence, in Zone B*b*, for a rapid climatic improvement before the opening of the classical Alleröd (as customarily defined by

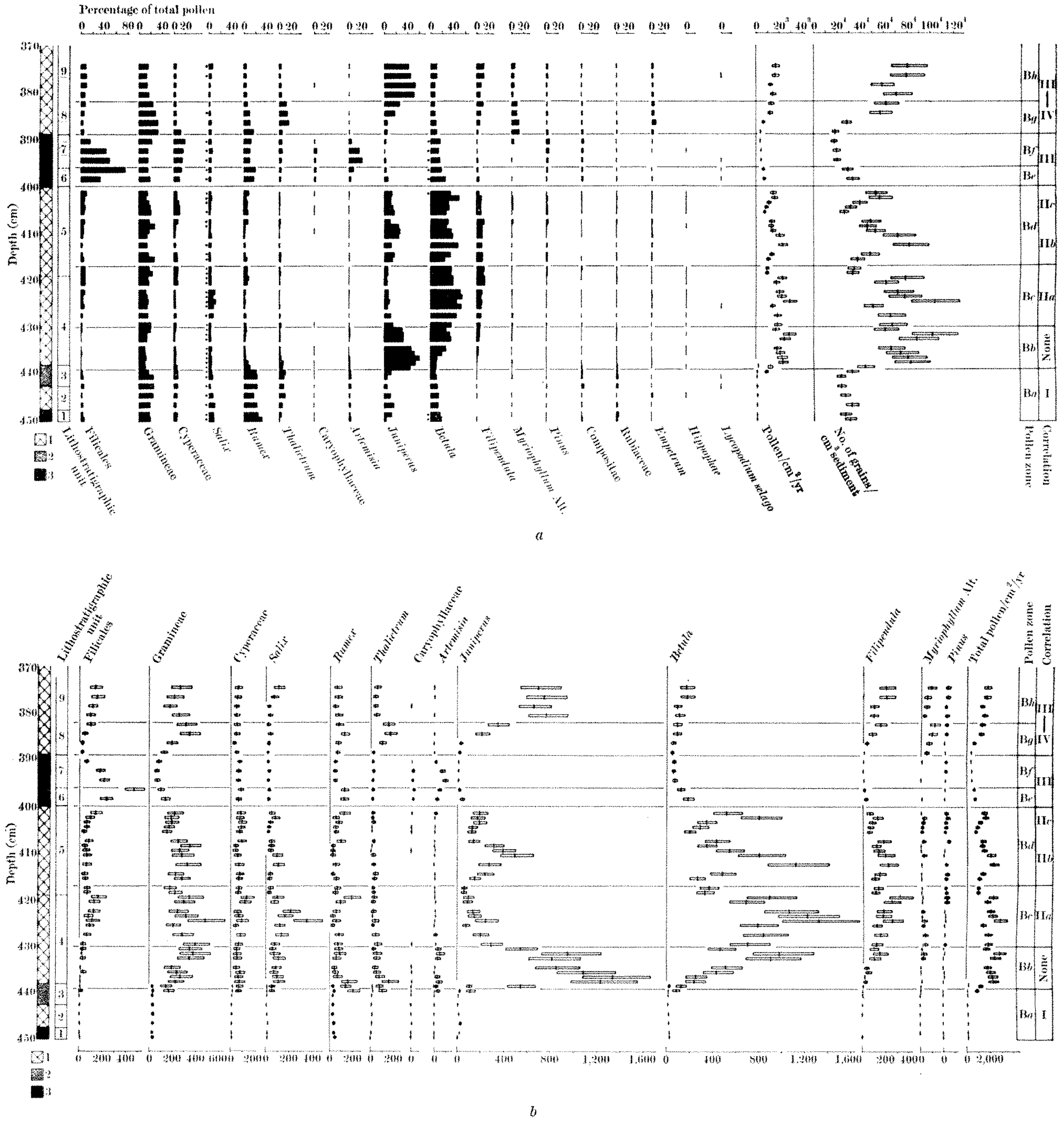

Fig. 2. Pollen diagrams from the 1968 profile from Blelham Bog. (*a*) Counts expressed as percentages of total pollen, together with plots of total numbers of grains, per square cm per year and per cm³, respectively. The pollen count for each sample was between 450 and 800 grains. The presence of *Betula nana* and of *Salix herbacea* is indicated by a cross on the left of the histogram for the genus. (*b*) Counts expressed as numbers of grains per cm² per year of the most important taxa. The plot for each value indicates the 90 per cent confidence interval for the counting error only. The count for each sample was 1,000 grains of fossil plus exotic pollen. Stratigraphic symbols: 1, organic mud; 2, silt of low organic content; 3, clay.

the rise in percentage frequency of tree birch pollen). This improvement, dated on our time scale to between 10,750 BC and 11,000 BC, is deduced from simultaneous changes in lithostratigraphy, in percentage pollen spectra and in pollen input per unit area per year. In none of these is there any evidence for recession between this amelioration and the tree birch period (Alleröd); on the contrary, the sequence can be interpreted as a continuous ecological succession. This reinforces the suggestion of Kirk and Godwin's dated pollen diagram from Loch Droma[14] that the onset of interstadial conditions began in western Britain early in the eleventh millennium BC, and that between this time and the height of the Alleröd interstadial there was no general climatic recession of sufficient amplitude to deflect the plant succession or to give rise to increased soil erosion. Our absolute pollen diagram provides evidence that the much earlier environmental fluctuation recorded in the stratigraphy of the Blelham Bog deposits was not accompanied by any significant change in pollen production by the local vegetation.

The sequence in this profile contrasts with that found at Danish sites such as Böllingsö[12,13], where a minor interstadial defined by the curve for tree birch pollen, Zone I*b* dated as 10,450 BC to 10,050 BC[9], is separated from the overlying Alleröd deposits by a minerogenic layer with little tree pollen, Zone I*c*. This indicates a regional difference between western Britain and Denmark in the lithostratigraphic sequence and in the pollen curves, which can most readily be explained as the result of a regional differentiation in Late-Weichselian climate.

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Fossil Ice in Hawaii ?

PERENNIAL ice was found late in 1969 near the summit of Mauna Kea, a dormant volcano rising 4,200 m above the sea on the island of Hawaii, 19° 49′ N and 155° 28′ W. A part of the ice-lava mixture lies close to the rocky surfaces near the bases of the south walls of the Goodrich Cone and Summit Cone craters (Fig. 1). Seismic refraction measurements have indicated a minimum horizontal extent of ice of hundreds of metres, and a thickness of many decametres. This ice may be the remains of known Pleistocene glaciation[1]. It is thought to survive near the surface in present climatic conditions because radiation effects make certain areas of the craters colder.

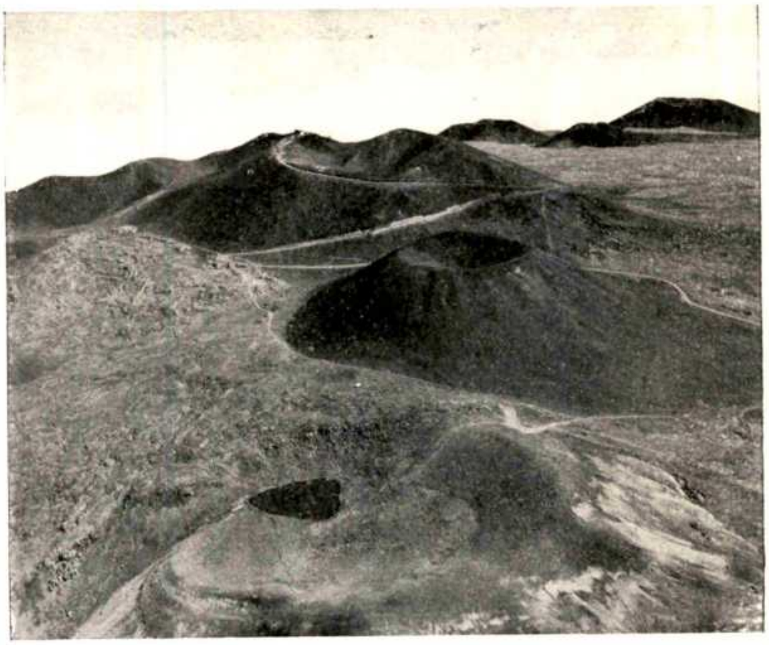

Fig. 1. Photograph of Mauna Kea summit looking north-north-east over Lake Waiau, which is in Waiau Cone crater, towards the Astrophysical Observatory of the University of Hawaii. The highest point, just to the right of the observatory, is immediately above the crater where the first old ice was found. Goodrich Cone crater, where more of the ice occurs, is to be seen between the lake and the summit. (Photograph by A. J. Abbott, Hawaii Institute of Geophysics.)

Carbon dating of Lake Waiau (Fig. 1) sediments[2] and temperature measurements in perched lake and ground waters[3], motivated by earlier geological and seismic exploration[1,4], led to the discovery of the ice. Brief examination at many locations revealed: (1) that the ice completely fills the interstices between the lava rocks and boulders; (2) the remains of insects imbedded in the ice; and (3) that the ice is clear, bubble-free, and tritium-free a few centimetres down.

Plans for further work concerning Mauna Kea include publication of seismic results, climatological studies in the craters, further seismic exploration, and drilling to collect deep ice samples. Evidence of climatic, biological and other conditions during Hawaii's remote past may be preserved in the ice.

Dr E. S. Deevey of Dalhousie University, Nova Scotia, Canada, helped at a critical stage in this study. Mr Theo Hufen of the Hawaii Institute of Geophysics made tritium measurements and Mr J. F. Campbell and Mr D. M. Hussong of the institute helped with the seismic work. The research was supported in part by the US Office of Naval Research.

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Bivalve-growth Patterning as a Method for Seasonal Dating in Archaeology

ARCHAEOLOGICAL evidence is often found for the exploitation of marine resources. In New Zealand, one of the most widespread species exploited was the intertidal bivalve *Chione stutchburyi*, and its growth dynamics form the basis of the technique described here, the purpose of which is to determine at what time of the year shells were collected.

Following the approach of other workers, studies of living populations of *Chione* indicated that, in general, the bands or rings on the surface of the shells (macro-rings) were the result of winter growth recession[1-4]. Further, when cross-sections of the shells were photomicrographed (Fig. 1) two major types of banding (micro-rings or bands) were observed—fine black bands and relatively broad white bands. The latter sometimes contain faint dark lines, indicating two growth phases between each black band. In a sample of twelve modern shells the number of major black rings between the two major winter rings varied from 300 to 420 with a mean of 358, suggesting that the black bands may be formed daily.

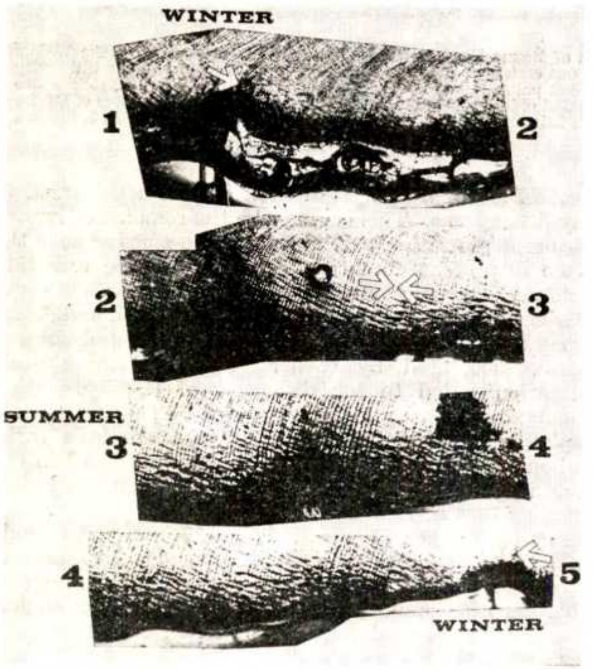

Fig. 1. Photomicrograph of a transverse section through the umbo of *Chione stutchburyi*. Daily micro bands are clearly discernible between the annual growth rings (macro bands). The latter are indicated in the photo by the vertical arrows. The horizontal arrows indicate the position of a pair of bidaily bands.

There is also considerable variation in the width of the bands throughout the year; they are very much wider in summer and spring.

An approximate estimate of the season of death of both modern and archaeological shell samples may be made, first by examining the variation in the thickness of the daily bands from the last macro-ring to the shell margin, and then by counting the number of daily growth bands from the last macro-ring to the shell margin. Provided that the approximate date when the macro-rings are formed is known, the date of death of individual shellfish may be estimated. For *Chione* this was determined by observing the growth dynamics of living populations over a period of twelve months.

A comparison between the known and estimated dates of death in twelve modern specimens (Table 1) suggested that an accuracy within a range of three months can be obtained.

Table 1. COMPARISON OF ESTIMATED DATES OF DEATH OF *Chione stutchburyi* WITH KNOWN DATES OF DEATH FROM THREE COLLECTING AREAS

Locality	Date of collection	Sample No.	Average No. of black bands from last winter rings to shell margin	Season of collection based on variations in the width of the bands
Area B Doctors Point	20/4/69	4	234 ± 20	Autumn
Area B Doctors Point	23/5/69	4	275 ± 20	Autumn
Tiwai Point	9/4/69	4	220 ± 15	Autumn

The method has been applied to shells from several coastal sites in New Zealand and the estimated seasonal dates, along with those obtained from other faunal data, are shown in Table 2.

Table 2. ESTIMATED DATES OF DEATH OF SHELLS FROM THREE ARCHAEOLOGICAL SITES IN SOUTHERN NEW ZEALAND COMPARED WITH SEASONAL DATES DEDUCED FROM OTHER EVIDENCE

Location of archaeological sites and stratigraphic position	Shell species considered	Average No. of black bands from last winter ring to shell margin	Estimated months during which shells were collected (by microscopic technique)	Estimated period of year when site was occupied from other faunal evidence
Tiwai Point TW PQ/40	*Chione stutchburyi*	110 ± 10	Dec–Feb	Oct–April
Tiwai Point P/30	,, ,,	90 ± 10	Nov–Jan	Oct–April
SP/1 TR B, sq 3, spit 1. Concentrated midden (SP = Southport)	,, ,,	146 ± 15	Dec–Mar	Nov–June
SP/1 TR C, sq 1. Concentrated midden	,, ,,	120 ± 15	Dec–Feb	Nov–June
SP/4 TR C and D, sq 2 and 3. Cockle	,, ,,	128 ± 25	Nov–Mar	Nov–June
SP/5 TR H, sq −5. Spit 2. Organic	,, ,,	101 ± 20	Dec–Feb	Nov–June
SP/5 TR E, sq, −7. Spit 2	,, ,,	103 ± 10	Dec–Feb	Nov–June
Wakapatu	*Protothaca crassicosta*	200 ± 20	Mar–April	Oct–April

Thus in New Zealand, at sites such as Tiwai Point and Wakapatu, the relatively thin midden deposits and presence of the bones of migratory birds suggest temporary occupation during the summer. In advancing a hypothesis that the sites were occupied at this time of the year for the exploitation of seasonal food sources, it must nevertheless be remembered that both fish and birds were dried and transported over fairly great distances during the protohistoric period in southern New Zealand. A means of checking this hypothesis is necessary. Although shells were also carried quite far during the protohistoric period in New Zealand, the number and weight of the shells that constitute the coastal middens in question rule out the possibility that they were transported to the sites from elsewhere. Shellfish were dried and stored during the protohistoric period[5], though there is no evidence that large quantities of the shells themselves were carried over any distance from the point of collection.

The implications of these results for the prehistorian are clear. The technique described provides not only a check but also a fairly refined estimate of the season at which the sites were occupied.

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Book Reviews

MYTH-MAKER

The Raw and the Cooked

Introduction to a Science of Mythology I. By Claude Lévi-Strauss. Translated from the French by John and Doreen Weightman. Pp. 387. (Cape: London, April 1970.) 65*s*.

In a recent edition of *The Times Literary Supplement*, Professor Longuet-Higgins drew a comparison between chemistry and cooking as both being activities concerned with experiments and observations on real substances. While regarding cooking as an admirable occupation he will not accept it as a science—"The difference is that chemistry is not an isolated study—a law unto itself—but part of an intellectual edifice designed to express our understanding of the whole material universe". Claude Lévi-Strauss would disagree with Professor Longuet-Higgins on this point, for the very foundation of *The Raw and the Cooked* rests on the assumption that cooking, its categories, its operations and its transformations, are the culinary code part of an intellectual edifice through which can be expressed not simply an understanding of a whole material universe but a whole spiritual one as well.

The Raw and the Cooked is the English translation of *Le Cru et le Cuit* which, as the first of the *Mythologiques*, appeared in 1964, and has since been followed by two further volumes with a fourth one promised. For the reader new to this science of mythology it is difficult to provide an adequate summary of this work. It is a wide-ranging comparative study of myth which starts with the Bororo Indians of Central Brazil, is chiefly concerned with the myths of South America, but does not hesitate to draw supporting evidence from societies situated in any time or place. This is permissible because of the fundamental unity of the human mind, which is partly what the exercise is concerned to demonstrate.

The difficulty of dealing with this work in a brief review, and even perhaps the impossibility of it ever being submitted to truly critical assessment, has been assured by the author: ". . . this book on myths is itself a kind of myth. If it has any unity, that unity will appear only behind or beyond the text and, in the best hypothesis, will become a reality in the mind of the reader" (p. 6). When, in 1955, Lévi-Strauss declared that Freud's Oedipus complex was simply an up to date version of the myth and no less a myth, one might have thought that he was being disparaging about the founder of psychoanalysis by casting him in the role of myth-maker. We can now see, however, that Lévi-Strauss meant this as a compliment, but it does mean that the structural analysis of myth, like psychoanalysis, is above criticism except through further analysis of the same sort.

This situation is worsened because critical access through the empirical evidence is also prevented. Lévi-Strauss carefully covers himself against the criticisms of the specialists in the ethnographic evidence which he has used, and he has found support in a reviewer of the third volume of the *Mythologiques* who claims that to worry about the factual base on which the whole science of mythology is built is to misunderstand Lévi-Strauss. This can only mean that facts may as well be dispensed with entirely, and indeed the ease with which Lévi-Strauss manages to fit what would appear to be contradictory evidence into his schema gives additional support to such a suspicion. One of the difficulties about a method which proceeds on a dialectic of oppositions and transformations is that almost any evidence can be incorporated if one is imaginative enough to think of the opposition, or tenacious enough to find one in the myths of the world.

The translators, John and Doreen Weightman, have made an excellent job of a very difficult task, and if passages do not seem to make much sense to the reader, it is not the fault of the translators but simply a reflexion of an opaqueness in the original. Having said this, one must add that the translation does Lévi-Strauss a disservice; in 1955 he stated that myth belonged to a linguistic pole at the other extreme from poetry in so far as the latter could not be translated without distortion while myth retained its value through the worst translation. It seems to me that the act of translating *Le Cru et le Cuit* refutes this because in the process it has been demythized. Freed from the myth-maker's language, the words lose their sacredness and the arguments become more glaringly defective.

On the other hand, with some experience of South American ethnography, I have found many of the ideas in this volume and the later volumes of the *Mythologiques* very enlightening, and have been amazed by the ingenuity and fertility of a mind which can make such improbable but farsighted connexions. There is no doubt that this book will sell on the author's name (the publishers recognize this by placing the author's name in letters four times larger than the title), but who is going to read it and who is going to understand it before it takes its silent place on the shelf alongside *The Golden Bough*?

Peter Rivière

TOWN LIFE IN AFRICA

Social Networks in Urban Situations

Analyses of Personal Relationships in Central African Towns. Edited by J. Clyde Mitchell. Pp. x + 378. (Manchester University Press: Manchester. Published for the Institute for Social Research, University of Gambia. November 1969.) 55*s*.

"Each individual in a society", one of the contributors to this symposium tells us, "has social relationships with a large number of friends and acquaintances. All these relationships together constitute what has been called a personal network." Starting from some such simple definition, and adding a few additional concepts, Mrs Wheeldon and two of the other contributors, Dr Epstein and Mr Boswell, are able to provide relatively straightforward and illuminating analyses of several previously unconsidered aspects of social life among one Coloured and two African communities in three different Central African towns.

The remaining contributors are much more ambitious in their use of the network concept. To understand fully their papers, the reader must master a sizable technical vocabulary that includes such terms as anchorage, reachability, density, range, content, directedness, durability, intensity, frequency, star, zone, cluster, boundedness, finiteness, reticulum and span. He may also be expected to have or to acquire some familiarity with mathematical graph theory and its sociological applications. Assistance in this considerable task is offered by Professor Mitchell who, in a paper that often draws heavily on the literature of American social psychology, reviews the meaning of many of the concepts and suggests ways in which they may relate to and improve upon earlier modes of anthropological analysis. Further help comes from Professor Barnes, whose paper develops the morphological implications of a social network far beyond anything that even he can have expected from it when he first introduced it in 1954.

Sophisticated applications and additional refinements of some of the concepts are provided in Dr Kapferer's analysis of the course and consequences of a dispute among a small group of African industrial workers in Broken Hill, and in Mr Harris-Jones's paper on the political meaning and significance of the ties arising from common rural origin amongst Africans living in the town of Luanshya.

It is noticeable, though, that in spite of the often close collaboration between the contributors, they do not always use the same words in the same senses. Such discrepancies make me doubt the practical wisdom, whatever the theoretical attraction, of bringing so many new terms into a situation where already, as Barnes writes, "each analyst reads a different interpretation into the writings of his predecessors and introduces new refinements to suit his own particular problem". I would complain, too, about some of the many proffered diagrammatic aids to the understanding of often complex arguments. Apart from minor, but irritating, faults in proof-reading such as the omission of one arrow and the reversal of another in Figure 1 (a), there are several tables and figures which are not self-explanatory and which it is the author's, not the reader's, job to elucidate.

Some of the conclusions to which these lengthy and elaborate arguments lead seemed suspect to me. How, for example, can Barnes possibly maintain that "under tribal conditions the denseness of the network leaves no room, as it were, for the addition of those idiosyncratic relationships of friendship with strangers, or even of patron and client, that helps to fill in the relatively sparse urban network" ?

The statement is contrary to fact, and rests on a dichotomy that seems increasingly untenable. I might, also, be more willing to accept Mrs Wheeldon's contention that "the many disagreements which are characteristic of Coloured social organization are not an indication of social disintegration; they are rather an aspect of the integration of a society of this kind", if there were not increasingly ample evidence that such disagreements in many African societies of an apparently similar kind can and do lead to schism and even violence on a scale which might legitimately be called "disintegration".

Still, the book is clearly, in many ways, one of the most remarkable anthropological contributions to urban studies that has ever appeared in Britain. It is likely to have a revolutionary effect both on those who wish for new ways of organizing their research, and on those who are eager to carry anthropology far beyond any traditional conceptions of its scope and methods. It has, too, a good deal to offer to those who are merely interested in Central African societies. W. J. ARGYLE

BACK TO DARWIN

Genetic Load

Its Biological and Conceptual Aspects. By Bruce Wallace. (Concepts of Modern Biology Series.) Pp. xi+116. (Prentice-Hall: Englewood Cliffs NJ and Hemel Hempstead, February 1970.) 60*s*.

THERE is a strange notion, derived from mathematical theories of evolution, that populations of organisms are threatened by a "genetic load", sometimes called the "cost of evolution". This idea looks fair to be for twentieth century evolution what phlogiston was for chemistry in another day; Professor Wallace's strength is that he plainly believes in oxygen.

Darwin said that the overproduction of organisms caused much dying, and that evolution occurs because early death is more likely for some than others (that is, natural selection). The theory of the genetic load goes into reverse gear and asks "As natural selection requires so much dying (a genetic load), how is it that organisms can evolve, or for that matter maintain their genetic variability, without becoming extinct ?" Wallace has a simple answer.

"Discussions of genetic load have too often failed to recognize the ecological aspects of population dynamics." Within wide limits a population absorbs new causes of death by a slight reduction in size; as the genetic load increases or decreases, it "is either drawn from or returned to the general ecological load of which it is a part", for most of the offspring produced must die in any event. If half the offspring die from genetic causes, that does not result in a population of only half normal size. But "the genetic load must not exceed the reproductive ability of [the] species". To account for the variability of natural populations, we must allow first that much of natural selection is "soft" or density-dependent, occurring only at high densities (a return to the original Darwinian idea); second, that the optimal genotype, with whose fitness that of the population is compared, is vanishingly rare in natural populations, so that the usual load theorem is unrealistic.

For the human population the story is different (Muller's idea that Man's genetic load might threaten the growth of his population would be funny if the position wasn't so tragic): as the genetic load means human misery, there is no excuse for throwing radioactivity around.

This argument, presented with great clarity (at the expense, it must be said, of some realism, but then the facts, about *Tribolium* for instance, are just too complicated), makes the third part of the book required reading for all geneticists and ecologists. An important question remains—how do populations avoid accumulating genetic variation that is maintained by "hard", that is, density-independent, selection ? Perhaps many don't; after all, there are more species extinct than extant.

The first two sections review the evidence for the genetic diversity of natural populations of fruitflies and men, and discuss some of the better known mathematics about the genetic load. Written with that transparent simplicity which is the mark of the assured master, they make an excellent introduction to these aspects of population genetics; any undergraduate who finds this mathematical treatment too much should not be reading biology.

The book is well produced, mercifully free of acres of Zipatone, and expensive. JOHN R. G. TURNER

ACCENT ON MAN

Évolution des Vertébrés

De Leur Origine a l'Homme. By Georges Vandebroek. Pp. xx+583. (Masson: Paris, 1969.) 120 francs.

As its title states, the aim of this book is to describe those parts of the pattern of animal evolution which are concerned with the line which leads from the higher metazoans to man. The existence of other lines and patterns of evolution is noted in the appropriate places, so putting the line to man in its context, but they are not dealt with in comparable detail. Professor Vandebroek, of the University of Louvain, concentrates on the points at which the line to man diverged from these other lines, because it was at these points that the various characteristics of man (vertebrate, tetrapod, amniote and so forth) were established. These matters are dealt with in a series of six chapters on the origins of the higher Metazoa, of the chordates, of the tetrapods (with special emphasis on the structure of the skull), of the mammals and of the eutherians (especially the primates). In each case, the evolutionary history of the parent group is described first, to form a basis for a more detailed discussion of the origin of the next, higher, group. The last three chapters are concerned with the mechanism of evolution and the concepts of species and of race, with the details of the origin of man himself, and with the present-day races and anthropological types.

These problems have interested Vandebroek for many years, and he has developed his own ideas on several matters. For example, he believes that Van Beneden's theory of the homology of the coelomic sacs of vertebrates with the mesenteric recesses of cerianthid coelenterates has been unfairly dismissed by modern workers. Vandebroek discusses this problem at length, with details of his own work, and concludes that Van Beneden was correct. Studies on gastrulation and the closure of the blastopore have further convinced him that the chordates are not related to the echinoderms and enteropneusts, but are more closely allied to the protostomes. This view in turn leads him to make comparisons between the circulatory and excretory systems of chordates and annelids based on the belief that they are basically homologous. Such views are highly controversial, and it is unfortunate that they have first been published in a book of this kind, rather than being first exposed to the winds of criticism and discussion by publication in a scientific journal. Vandebroek also believes that the monotremes are descended from the Permian dicynodont reptiles. Finally, he has elaborated a new theory on the homologies and evolution of the molar cusps in mammals, together with a complete new terminology for these cusps. This theory is thoroughly presented in his book, with the aid of a large number of excellent figures and photographs of the molar teeth of mammals. Published in 1961, his theory has not so far received any measure of acceptance from other workers in this field.

It is clear that Vandebroek's book is not suitable for the newcomer to this field of enquiry, who requires an explanation of the generally accepted view on these matters which he can then elaborate by reference to other textbooks. It will instead be of interest to the proponents of the "generally accepted view" who may wish to test it against the theories proposed by Vandebroek and, in the process, criticize his theories in their turn.

The book is extremely well produced, with many new and excellent figures and photographs. In contrast, the reader who wishes to consult the original papers which have influenced Vandebroek's thinking will unfortunately find, by way of a bibliography, merely a list of forty-six general textbooks. BARRY COX

DEVELOPMENT OF MALIGNANCY

The Cancer Problem

A Critical Analysis and Modern Synthesis. By Armin C. Braun. Pp. ix + 209. (Columbia University Press: New York and London, February 1970.) \$8.50; 76*s*.

IN cancer research, it seems that a disproportionate amount of attention has been given to the growth of malignant cells *per se*, compared with normal cells, and tissues. This very good book forces one to put malignancy in a much more meaningful perspective within the framework of cell and developmental biology. The central thesis is that the tumour problem is what controls the growth of normal cells and where this control breaks down in neoplasia. Metastasis and invasiveness are regarded as much less important. In approaching this problem, the author takes essentially an epigenetic view of cancer, arguing for the equivalence of the malignant state with that of a differentiated state: whatever enables cells to acquire heritable changes during development is essentially the same as the development of malignancy.

The books starts by lucidly reviewing the basic biological concepts that underlie approaches to the tumour problem, including a consideration of viruses, genetic influences and the essence of biochemical hypotheses. He gives very little attention to carcinogenic agents or X-rays because, "while they are important for initiation of tumour they play no part in the continued abnormal growth of the tumour cells". He then goes on to consider the regulation of cell division and the capacity of autonomous growth. This leads him to the nature of heritable cellular changes, and whether somatic mutation is an essential prerequisite. He concludes from consideration of viral transformation and the nature of differentiation that ". . . we have in the concept of active and inactive gene states within a constant cellular genome a very real and, above all, an experimentally testable alternative to the concept of somatic cell mutation as an explanation for the heritable cellular change that underlies the tumorous state generally". He also argues that there is evidence that the malignant state may be a potentially reversible event. This brief summary does not do justice to the large amount of information from a variety of systems, including plants that he so draws upon and describes.

In considering the normal and abnormal growth of cells, and in regarding the essential abnormality of tumour cells as their continued or autonomous growth, the author repeatedly expresses the view that, for a cell, the changes leading to cell division involve a radical reorientation in biosynthetic metabolism. This is based on the idea that for cell division much of the cell's economy must be directed towards the synthesis of mitotic spindle proteins, which he suggests may comprise up to 30 per cent of the cell's total protein, and is almost certainly wrong. Current estimates of the amount of microtubule protein in the spindle put it no higher than 0·2 per cent of the total protein. While this does not at all invalidate the main lines of argument in the book, it highlights its weakness: in attempting to interpret cancer within the framework of the control of cell division and differentiation of normal tissues, it is a bit like explaining the unknown in terms of the unknown. It is not, for example, just that our current ignorance of the control of cell division is profound, but also at the conceptual level the discussion of the problem is still confused. We do not have a clear image of how we should think of the intracellular control of cell division which is the paradigm for coordination within the cell. Mitotic operons provide a useful shorthand for concealing our ignorance when considering intercellular mechanisms, involving, for example, chalones, but even here our ignorance is striking. The situation *vis-à-vis* differentiation is not much better and the author is unfortunately too uncritical of the old concepts of embryonic organizers, fields and a plethora of inducing substance. I think I could make a strong case that organizer-inductor ideas were a conceptual error with near disastrous consequences for developmental biology.

The great value of this book lies in bringing together critically, clearly and briefly, within the current framework of cell biology, some of the essential features of the cancer problem. Its weakness is that the organizational problems of cell growth and development in terms of cell and molecular biology still elude us. But it gives hopes that this will not be for long. LEWIS WOLPERT

PERSONAL ENERGY

Energy and the Mitochondrion

By David E. Green and Harold Baum. Pp. xii + 205. (Academic Press: London and New York, January 1970.) 79*s*.

IT is difficult to disparage a book by an eminent researcher, but in all humility I feel I must take an exception to this misleading tome.

If one wishes to know how Professor Green currently views the field of mitochondrial and membrane research and of oxidative phosphorylation, this is the book to read. This is substantiated by the fact that two thirds of all the "special articles" references are from his laboratory, and by the preface stating that "we have restricted our presentation to the point of view to which we have been led by our experimental findings". The authors further state that "Notions and concepts that we believe no longer have validity have been passed over in silence"—in this brief sentence a large base of information is thus conveniently jettisoned.

To find out how Green and Baum interpret the universality of membrane conformational changes one needs only to consult the front cover and a figure (p. 58) which explain the phenomena of "nonenergized", "energized" and "energized twisted" states as the conformational cycle of the tripartite repeating unit—this latter entity is considered the key to all membrane phenomena. The electron micrographs of isolated mitochondria showing the various conformational states are recognized facts, but the intricate diagrams derived from these, which form the basis of this book, should, in my opinion, be considered very, very tentative interpretations and definitely not "a logical, integrated interpretation of the mitochondria as an operational and transducing unit". This is, in fact, one view of an overall system which may represent primary, secondary or possibly even artefactual phenomena.

In the last chapter, the authors try to extend their thesis to other energy transducing systems, ranging from pinocytosis to photosynthesis and including vision and muscular contraction. While I do not doubt the universality of some membrane conformation changes, this attempt at universality is stretching things a bit far. For example, the author's claim of a role for the tripartite repeating units in photosynthetic electron transport and phosphorylation is tenuous in the extreme—the evidence is hotly contested, and the inclusion in the book of unpublished work from Green's laboratory does not help matters. The electron flow schemes presented are also of dubious validity. Similar criticisms can be made for deductions on the process of visual excitation and amplification in rod outer and inner segments of the eye.

A concluding postscript chapter purports to lead "to a great simplification of the configurational picture" by now postulating four possible configurational states in all mitochondria. The evidence is derived from six unpublished papers, which is asking much, except from those already convinced.

An appendix has a very useful compilation of many formulae of compounds of interest to workers in the field of mitochondrial research.

I do not feel that this highly personalized book can be recommended for general students in biological fields (as is claimed on the flyleaf), but it may be of interest to specialists in the field who wish to follow the current lines of thoughts and research in the authors' laboratory.

D. O. Hall

STEROIDS AFTER NINE YEARS

Steroid Biochemistry

By Erich Heftmann. Pp. xiii + 220. Academic Press: New York and London, December 1969.) 89*s*.

In 1960, Heftmann and Mosettig wrote *Steroid Biochemistry* as an introductory text for plant and animal biochemists and physiologists, endocrinologists, pharmacologists and clinicians. The enormous increase in knowledge of steroids has rendered this book out of date, and Dr Heftmann has undertaken the daunting task of rewriting it in 26 pages less than the original. Organic chemistry has been kept to a minimum and only occasional use of jargon (hydride shifts) will upset the uninitiated. An attractive feature of the book is the wonderful panoramic view which it gives of steroid biochemistry. In some medical circles steroids mean little more than the adrenocortical steroids and their synthetic analogues. Here we are introduced to the rare and exotic steroids, without known function, which are found in plants and lower forms of animal life. It will come as a surprise to many that some of the well known mammalian steroid hormones are produced and metabolized in plants. There are fascinating references to the secretion of testosterone by water beetles, which have been used as aphrodisiacs in folk medicine, and steroid cardiac poisons in primitive trials by ordeal. Steroid analogues, drugs and inhibitors, which have found use in academic and clinical work, are mentioned with their structural formulae.

The author naturally does not attempt to refer directly to the original vast literature. He recommends the use of the index (16 pages), which correlates text pages, structural formulae and references in a bibliography of 692 books and reviews. The bibliography contains references to items in Russian, middle European and Scandinavian languages with titles translated. One wonders if the author has personal knowledge of the content of such articles and thus of their worthiness. This form of reference is perhaps the best one can expect in a small book, but it is often frustrating to be unable to trace the source of some statements. One would, for instance, like to check the reputed reduction of deoxycorticosterone to pregnanediol in man, and wonders if indeed it is not misleading even to mention this when so little space can be given to description of metabolism. Similarly, it would seem unwise to mention the alleged occurrence of equilin in an adrenal tumour without qualifying reference to evidence of identification. Predictably, there is no space for the description of experimental results which led to important discoveries.

The book is perhaps weakest in the sections dealing with endocrinology of steroids. One looks in vain for mention of recent work on the action of 5α-dihydrotestosterone. The description of the action of androgens in the foetus and neonate is not clear; nor is the reference to enzyme induction by androgens. One would prefer some reference to chemical and physical methods of measuring steroids rather than bioassays. The concentration of plasma oestrogens of less than 1 mg per 100 ml. is surely a printing error.

These criticisms are admittedly of rather a carping nature—one enjoys reading the book so much. The bibliography and the numerous structural formulae will undoubtedly prove most useful.

J. K. Grant

MOLECULAR CYTOLOGY

Handbook of Molecular Cytology

Edited by A. Lima-de-Faria. (North-Holland Research Monographs Frontiers of Biology, Vol. 15.) Pp. xv + 1508. (North-Holland: Amsterdam and London, 1969.) 560*s*.

The handbooks published by the Chemical Rubber Company are invaluable reference works; that the *Handbook of Chemistry and Physics* is now in its fiftieth edition and has seven stable mates speaks for itself. Unfortunately, anybody anticipating that North-Holland's *Handbook of Molecular Cytology* might resemble and be as useful as the CRC handbooks is in for a great disappointment. To be sure, this so-called handbook is large and expensive, but there the parallel with the CRC's publications ends. The *Handbook of Molecular Cytology* is nothing more than yet another collection of inevitably outdated review articles. It resembles nothing so closely as a bumper edition of *International Reviews of Cytology* or a one volume condensation of Brachet and Mirsky's *The Cell*.

This book is the fifteenth volume in the publishers' series "Frontiers of Biology" but differs from its predecessors in not being devoted to a restricted topic; it attempts to cover the whole of modern cytology and suffers as a consequence. Lima-de-Faria has gathered together a group of contributors which is distinguished enough, but for the most part all too familiar, with the result that the overriding impression the reader gets is of having seen the book's contents before elsewhere.

The section on chromosome organization, for example, begins with an article by Ris; Britten, McCarthy and Walker and his colleagues all have their say on satellite DNAs and the evolution of DNA base sequences; Bernhard writes on the ultrastructure of cancer cells, Callan on lampbrush chromosomes, Afzelius on cilia and flagella, Bennett on the cell surface, Robertson on the structure of membranes, and so on through the list of fifty-three contributors. The organization of the book is equally familiar. It starts with a section on the evolution of DNA and then progresses first to the organization of viral, bacterial and eukaryotic chromosomes, followed by the structure and bichemistry of the interphase and dividing nucleus, and ends up with the cavalcade of the cell organelles. I admit the volume is not entirely without surprises. The editor deserves to be congratulated, for example, for deciding to include articles on abnormalities of sex chromosomes and autosomal chromosomes, and for persuading Hamerton and Taylor, both from the Paediatric Unit at Guy's Hospital, to write them.

Books of this sort are always out of date before they are published; in this case, as far as I can see, the most recent references date from early 1968. And inevitably it is incomplete; somatic cell genetics, tumour virology and the work stemming from Watkins and Harris's discovery of virus mediated cell fusion are all topics one might expect to find discussed but they are barely mentioned.

The blurb talks of the handbook being the result of "the pressing need for a deeper understanding of the molecular interactions within cells" and of our being close to the threshold of some great synthesis that will provide an all-embracing view of the cell. The need for a deeper understanding of cells is obvious, but I doubt if this book will help much in bringing it about. Of course it contains many useful pieces of information but they are far from being in that particularly accessible form one expects of something parading as a handbook. Accessibility of information is precisely what has made the Chemical Rubber Handbooks so successful and useful. The *Handbook of Molecular Cytology* is not in the same category in spite of its title, and purchasers, even libraries, have a right to expect a great deal more than this tome offers if they are being charged £28.

JOHN TOOZE

NUCLEAR REACTIONS

Nuclear Reactions

By I. E. McCarthy. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Selected Readings in Physics.) Pp. viii + 327. (Pergamon: Oxford, London and New York, February 1970.) 45*s* ($7) boards; 35*s* ($5.50) flexi-cover.

THIS book is designed to introduce the theory of nuclear reactions to undergraduate students after a first course in quantum mechanics. The first part of the book contains a 100-page review of the theory of scattering and reactions, and is followed in the second part by a selection of original papers published between 1929 and 1965.

The introductory review covers the elements of scattering theory, the theory of the compound nucleus, the optical model, a discussion of nuclear structure and nuclear forces, and a discussion of direct reactions. The wide range of subjects and the condensed form in which they are described seem to make this section far too difficult for undergraduates. For example, the basic scattering theory, including the definitions of differential cross-section, partial wave expansion, resonance and the like, is presented without any diagrams at all, the physical significance of the asymptotic form of the wave-function is not discussed, and such terms as phase shift and channel radius are not explained. Later on, the description of compound nucleus theory is much more advanced than that usually given in undergraduate texts, and the discussion of nuclear structure includes the Bethe–Goldstone equation and the non-local K-matrix. It is doubtful whether this part of the book would be of any value to postgraduates, because those who are specializing in nuclear reactions would need a more detailed treatment of the subject and those who are reading for general interest would find the explanation of the physics rather inadequate.

The choice of original papers by Rutherford, Mott, Bohr and Serber seems ver appropriate, but the other papers chosen are much too demanding for undergraduates. While one can agree with the publisher's contention that undergraduates should not only learn from textbooks but should make acquaintance with the original literature, it will be cheaper and more profitable in this case to send the students to the library.

DAPHNE F. JACKSON

BUBBLE CHAMBERS

Cloud and Bubble Chambers

By C. Henderson. (Methuen's Monographs on Physical Subjects.) Pp. vii + 118 + 4 plates. (Methuen: London, January 1970.) 38*s*.

THE title of this book is not altogether appropriate: it is largely about bubble chambers, their application to accelerator physics and the processing and analysis of bubble chamber photographs. References to cloud chambers are not in comparable detail, are uneven and are on the whole slanted to their possible application in the same area: the discussion of cloud chambers provides no guidance at all to the sort of problems recently set by possible "quark" tracks from Sydney! The author is probably not as at home in the cloud chamber literature as in that of bubble chambers: for example, diffusion chambers of the Langsdorf type were first described in 1936, not 1939. If, as is probably intended, the cloud chamber material is provided for purposes of comparison of the nucleation process and principally as a background to bubble chamber technology, it would have been valuable to give also comparable data and discussion on the scope and usefulness of nuclear emulsions and the problems of track measurements in them, and perhaps a brief if wider survey of the whole field of application of "visual track" detectors of all kinds.

Subject to these reservations, which are really substantial only as regards the title, this is an excellent book which provides a concise, well written and readable background to the technology of bubble chambers used with large particle accelerators. It brings out clearly the problems of construction of the chambers themselves and of expansion systems, and the varying conditions and purposes for which different liquid fillings are suitable. The differences in bubble development in various liquids are treated admirably, and for the non-expert reader the very different optical problems in hydrogen and in heavy liquid chambers, and some of the elegant solutions of these problems, are some of the unexpected features which emphasize the practical approach in a very well balanced monograph.

The book gains from exceptionally well chosen and reproduced photographs, which are set out with excellent descriptive material. It should appeal to a very wide range of readers.

J. G. WILSON

ANALYSIS WITH ENZYMES

Enzymatic Methods of Analysis

By George G. Guilbault. (International Series of Monographs in Analytical Chemistry, Vol. 34.) Pp. xv + 347. (Pergamon: Oxford, London and New York, January 1970.) 75*s*; $10.

THIS book, which contains a relatively brief survey of some aspects of the use of enzymes in analytical chemistry, manages to convey a large amount of misinformation. The first chapter deals principally with enzyme kinetics, but only deals with single-substrate kinetics and makes no mention of enzymes which do not obey simple hyperbolic kinetics. Inhibition is, justifiably, divided into reversible and irreversible types, but the example of irreversible inhibition given is non-competitive inhibition, and the reader is told that: "In reversible inhibition, the enzyme can recover its activity when the inhibitor is removed; in the case of an irreversible inhibitor it does not." Later on in the book there is a figure implying that while an irreversible inhibitor will yield complete inhibition of an enzyme if a sufficient amount is added, a reversible inhibitor will never cause complete inhibition. We are also told that the international unit of enzyme activity is measured at 25°, although the temperature for such determinations has been fixed at 30° since 1964.

It would take too much space to give a complete list of the errors and misleading statements contained in this book, and it may be that the publishers could consider offering a prize for the reader who can spot the greatest number of errors. A few examples taken from other chapters in the book should, however, give an idea of the troubles. The chapter on the determination of substrates tells the reader how to determine DL-succinic acid, and advises the use of malate dehydrogenase for the determination of adipic, DL-citric and D-tartaric acids, although this same chapter contains a table indicating that none of these three acids are substrates for malate dehydrogenase. This table illustrates the remarkably low specificity of the commercial preparation of malate dehydrogenase used by the author, but no mention is made of the considerably higher specificity of highly purified preparations of this enzyme.

This book contains brief discussions of some of the less common methods of enzyme assays and gives enthusiastic accounts of some of the more devious coupling methods that can be used to increase the sensitivity of enzyme assays, but, because of the numerous errors, it represents a potential hazard to any unsuspecting student hoping to learn about enzymes and their uses. K. F. TIPTON

Obituaries

Professor H. H. Read

HERBERT HAROLD READ, FRS, died on March 29 at Whitstable, Kent, not far from the farm where he was born in 1889. Trained in geology under Watts at Imperial College, Read joined the Geological Survey of Great Britain in 1914 and served in Scotland for seventeen years interrupted only by three years of war service. In 1917, Read married Edith Browning who, with their only daughter, survives him. His Geological Survey work resulted in accounts of the layered basic intrusions in north-east Scotland, in extensive descriptions of migmatite complexes in the Northern Highlands and in Shetland, in substantial contributions to Dalradian stratigraphy and in the recognition for the first time of several important phenomena connected with metamorphism.

In 1931 Read succeeded P. G. H. Boswell in the chair of geology at Liverpool and subsequently followed Boswell once more to the chair of geology at Imperial College in 1939. In the same year Read was elected a Fellow of the Royal Society and in September was to preside over Section C of the British Association, though his address on metamorphism and igneous action was never delivered because of the outbreak of war. Publication of this address and of seven others which followed over the next fifteen years, including those given as president of the Geologists' Association (1942–44) and of the Geological Society of London (1947–49), established his international reputation. It is not easy to summarize the content of these addresses; leaving details aside, Read might be said to have attained two objectives. He set out to show how granitic rocks are distributed through the crust, paying particular attention to the role of granites in migmatite complexes, and to demonstrate that the non-fossiliferous crystalline rocks preserved a record of their geological history, which could be incorporated in the stratigraphical record already established through the study of sediments.

Read presided over the International Geological Congress held in London in 1948. In the same year he began his most extensive single investigation when, in collaboration with Professor R. M. Shackleton and Professor W. S. Pitcher and other colleagues, he started a survey, now successfully completed, of the crystalline rocks of Donegal, the results of which are set out in numerous papers. His researches led to his election to the National Academies of Belgium, France, Ireland and Norway, to honorary membership of many geological societies, and to the award of the Geological Society's Wollaston Medal and in 1963 of a Royal Medal from the Royal Society.

Read wrote with facility and was a masterly lecturer. As well as his four Geological Survey memoirs he wrote either alone or in collaboration with Janet Watson, five books and had a sixth in an advanced stage of preparation at the time of his death. In a review published in 1949 (*Geogr. J.*, **114**, 214), W. J. Arkell described Read's *Geology: An Introduction to Earth History* as a work of genius. This book epitomizes Read's ability to write clearly, attractively, often light-heartedly, and yet to convey a sense of awe. The final words of Arkell's review with his quotation from Read may be left to speak for themselves: "If by the time he reaches the last page the reader cannot enter into the spirit of the peroration: 'The geologist, standing on the earth as upon a footstool, looks at the past with wonder and turns to the future with hope', it will not be through the fault of the author".

Correspondence

Rhythm of Rabbit Teeth Cells

SIR,—Discussing the findings of Dr H. P. Rush (*Nature*, **226**, 406; 1970) that DNA synthesis in the slime mould *Physarum polycephalum* occurs immediately after mitosis and without an intervening G_1 period in the cell cycle, your correspondent suggests that most of the available data of this kind relate either to artificial situations, to slime moulds or to simple unicellular forms such as amoebae.

This is not entirely the case, however. In the proceedings of a symposium on naval medicine held at the Royal Society of Medicine in 1965 I reported that in rabbits the pre-ameloblasts (specialized cells concerned with laying down enamel matrix on the continuously growing teeth) have a cell cycle occupying between 19 and 20 h which was summarized in the following terms:

$$\begin{aligned} G_2+M+G_1 &< 10\text{ h} \\ G_2+M &> 9\text{ h} \end{aligned}$$

In other words, the duration of G_1 is very short, and synthesis must begin almost immediately after mitosis is complete[1].

Yours faithfully,

WILL STARKEY

MRC Dental Unit,
The Dental School,
Lower Maudlin Street,
Bristol BS1 2LY.

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[1] Starkey, W. E., *J. Roy. Nav. Med. Serv.*, **51**, 193 (1965).

Reprint Request Reflexes

SIR,—When you accepted my letter (*Nature*, February 14, 1970) you asked me to let you know the result. Especially as you published a similar letter adjacent to mine, I suspect that I may be taking part in your experiment, which I do most willingly! My experiment was of the "try it and see" variety. The interim result, for the first period of nearly two months, is as follows.

The most gratifying result to me was receiving a number of appreciative letters from colleagues. So far, there have been twenty-one request cards from USA and Canada and twenty-one from other countries. The number from USA and Canada might well have been higher but for the gratuitous republication of our letters in the advertising paper *Dateline in Science* of March 6, which I suppose is widely distributed among pharmacologists and others with an interest in prostaglandins. In any case, Letters to the Editor may possibly attract fewer requests than main articles.

In the meantime, requests for the original article continue to come in and must now number more than a thousand. All are from biologists although the article was written for non-biologists. It seems that I much underestimated its popularity, even if one allows that the number of spurious requests was much greater than the forty-two suggested by the "experiment" so far. Even this number is big enough to make me think I should add at the end of my next paper "Reprint requests by personal letter will be met while supplies last". The alternative would be to ignore the lot, except for those from places where the journal might be unavailable.

Yours sincerely,

V. R. PICKLES

University College,
Cardiff.

SIR,—We shared Professor Pickles's experience of receiving remarkably few reprint requests to our spoof letter (*Nature*, February 14, 1970). So far, there have been five or six from Europe, two from the United States and assorted requests to know the result of the experiment or to have a reprint, while pointing out that they had read the letter. Perhaps people in the latter category acquire all of *Nature* by reprint request rather than paying 4*s* weekly. An interesting request, and one which we find not uncommon, is for the wrong reprint. In this case we were asked for a paper in *Biochemical Journal*, and we can only surmise that an unfortunate secretary has slipped a line on a print-out and sent the wrong request to hundreds of authors.

We agree that republication in *Dateline in Science* did our cause no good and are sorry that what we regarded as a delicate flower which would take a month or two to bear fruit had to be pulled up by its roots in an early stage to fill a spare column and receive a withering blast of publicity.

But perhaps we are pessimistic about the all-rounder. Perhaps child psychiatrists are interested in the development of the Red Sea.

It has occurred to us that the words "Plate" and "Red" in a recent paper[1] may afford us a chance to catch the haematologists. We are only sorry now that we did not make the title "Plate Tectonics of the Red Sea; an Embryo Ocean".

Yours sincerely,

D. DAVIES

MIT E-34,
42 Carleton Street,
Cambridge, Massachusetts 02142.

D. P. MCKENZIE

Department of Geodesy and Geophysics,
Madingley Road,
Cambridge, England.

J. S. TURNER

Department of Applied Mathematics and
Theoretical Physics,
Silver Street,
Cambridge, England.

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[1] McKenzie, D. P., Davies, D., and Molner, P., *Nature*, **226**, 243 (1970).

Are More Airports Necessary ?

SIR,—A recent editorial comment (*Nature*, April 25, 1970) suggests that the advent of the 300 m.p.h. hovertrain "may be the signal to banish the new airport once and for all from the heavily populated areas around London". When the full implications of high-speed surface transport are taken into account we may be able to enlarge the point with economy of words by stopping at the phrase "once and for all".

Let us first consider the effects of a rapid-transit line connecting the airports of Gatwick, Heathrow, Birmingham, Manchester, Prestwick, Abbotsinch and Edinburgh, with the additional assumption that these airports (and, perhaps, other convenient stations on the high-speed spine) would be connected to city centres by intermediate speed (say 100 m.p.h. train) collecting links.

The total length of the main line (which could follow existing motorway routes for much of its length) would be about 480 miles, corresponding to a non-stop journey time of about 1·75 h from one end to the other. Given a peak acceleration of $0{\cdot}1g$, total acceleration time from a standing start would be about 3 min, and each 2-min stop would therefore add about 5 min to the journey. If the whole line had eight or nine stations on it, the journey time from end to end would be about 2·5 h, and an average journey might take a little over an hour. These figures are seen to be comparable with road or conventional rail journeys from London to any of Roskill's four sites.

The immediate effect of such a system would be to eliminate the need for the great majority of internal flights, and to permit overseas flights to be redistributed between airports. At present, flights between the airports mentioned account for about 12 per cent of the London load, and this figure can be doubled for the UK as a whole because there is an aircraft movement at a provincial airport corresponding to every domestic flight entering or leaving London. If this ratio is maintained as London traffic expands, we see that four-runway capacity in the London area ties up a total of one runway in the UK on domestic traffic. Eight-runway capacity for London ties up two UK runways. The traffic figures for the other airports on the list indicate spare capacity, by London loading standards, of at least three runways. The case for adding four new runways in the London area thereby loses its validity.

The foregoing case has been based on the gross capacity of only seven airports, but the actual reserve in Britain is far greater than this. The 1968–69 report of the British Airports Authority lists twenty-seven mainland airports carrying year-round scheduled services, and suggests that sixteen of these (excluding the third London airport) would be viable in the 1980s. In fact, most of these would be considerably under-used on the Authority's assumptions, but several could be linked to our transport spine to provide reserve or additional scheduled capacity.

The use of high-speed surface transport would reduce the problem of scheduling interconnecting flights from the various airports to about the level of that which was previously envisaged for the London airport system. The increased geographical dispersion would greatly improve the options of flight diversions to avoid unfavourable weather conditions and would also reduce the problem of air space congestion. By extending the transport spine to the point of entry to the Channel Tunnel, the problem of dispersing surface traffic from Europe would be alleviated, and if the system could be extended into Europe the airport pressure resulting from European traffic would be reduced.

On the basis of the suggested capacity for the third London airport, it would appear that the transit system should be designed for an ultimate capacity of at least 100 million passenger movements per annum. In the proposal for a tracked hovercraft, each vehicle is taken to have 200 seats. Taking an average journey lasting one hour, and assuming seat utilization of 2,500 h per annum, the number of passenger seats needed is 40,000, and about 200 vehicles are required. These vehicles, operating on a five-hour round trip, could provide peak service at intervals of about 1·5 min in each direction. Safety requirements would, perhaps, require the individual vehicles to be grouped into trains under these maximum-density conditions, but this further step would mean that even higher passenger flows could be sustained if necessary.

We now see that we have the beginnings of a transport hierarchy which can be extended downwards to connect with urban services. If such a hierarchy is built up on the principle of dynamic similarity between levels (the same peak acceleration, and similar journey shape), the time required for each stage is proportional to maximum veolicity, and the length of each stage is proportional to the square of the velocity[1]. We can also show that the rate of acceleration through the hierarchy (ignoring time lost in changing vehicles) is of the form

$$a = a_0(R-1)/R^2$$

where R is the speed ratio between two stages and a_0 is a measure of the acceleration in any one stage. Thus $R=2$ is the theoretical optimum on the elementary theory, but would be inconvenient in practice because of the excessive number of changes. $R=3$ seems a reasonable compromise.

On filling out the hierarchy in this way, we would envisage pedestrians boarding local buses to accelerate to about 10 m.p.h., and these serving high-speed buses or local rail feeders moving at 30 m.p.h. Theoretical average stage lengths would then scale as follows:

300 m.p.h. hovertrain	90 miles
100 m.p.h. feeder train	10 miles
30 m.p.h. coach or local train	0·9 mile
10 m.p.h. local bus	0·1 mile

Considerable adjustment of actual stage length to suit the location of pick-up points can be accommodated without much loss of efficiency. We should recognize, however, that the concept of feeding aircraft with conventional surface vehicles (say 40 m.p.h.) implies a value of about 15 for R, so that the present painfully slow acceleration from walking pace to air speed reflects a gross design error in the conventional system.

In all, we see that the hovertrain opens possibilities for a massive reorganization of the worldwide pattern of passenger transport, whilst demanding little more than minor new development at the other levels of the hierarchy. Doubts about its commercial viability therefore seem strangely myopic.

Yours faithfully,
P. L. WILLMORE

International Seismological Centre,
Edinburgh.

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[1] Willmore, P. L., *Architectural Rev.*, **142**, No. 847, 189 (1967).

Announcements

University News

Professor E. N. Hiebert, University of Wisconsin, has been appointed professor of the history of science in **Harvard University. Dr Clement B. Sledge** has been appointed professor of orthopaedic surgery at Harvard. He will be chief of the orthopaedic service at the Peter Bent Brigham Hospital and at the Robert B. Brigham Hospital. **Dr F. M. Ingersoll** has been promoted to clinical professor of gynaecology, and **Dr M. D. Schulz** to professor of radiation therapy at the Massachusetts General Hospital.

The title of Professor of Chemical Pathology has been conferred on **Dr F. V. Flynn,** in respect of his post at University College Hospital Medical School, **University of London.**

Dr G. P. Dinneen has been appointed director of the Lincoln Laboratory, **Massachusetts Institute of Technology,** in succession to **Dr M. U. Clauser** who has resigned to become academic dean of the **US Naval Postgraduate School** at Monterey.

Appointments

Professor J. W. L. Beament, University of Cambridge, and **Professor R. W. Edwards,** University of Wales, have been appointed members of the **Natural Environment Research Council.** Professor Clapham, Professor Lighthill, Sir Maurice Yonge and Sir Frederick Bawden will retire from the Council on May 31, although Sir Frederick will continue to attend meetings in his capacity of assessor for the Royal Society.

Miscellaneous

Applications or recommendations for the **Corday-Morgan medal and prize** are invited by the Council of the Chemical Society. Three awards for experimental work in different branches of chemistry will be made for 1969, each consisting of a silver medal and a prize of 250 guineas. Candidates should be British and under the age of 36 when the work was published. Further details can be obtained from the Scientific Affairs Officer, Chemical Society, Burlington House, London, W1V 0BN.

The Trustees of the **Beit Memorial Fellowships for Medical Research** have elected the following junior fellows: **C. T. Kirkpatrick,** to work at the Queen's University of Belfast; **Y. K. Levine,** to work at the MRC Molecular Pharmacology Unit, Cambridge; **W. Montague,** to work at the University of Sussex; **A. C. R. Samson,** to work at the University of Leicester; **M. A. Chester,** to work at the Lister Institute of Preventive Medicine; **G. E. Morris,** to work at the University of Sussex; **D. J. Warren,** to work at the Radcliffe Infirmary, Oxford; **M. T. Wilson,** to work at the University of East Anglia; **M. R. C. Winter,** to work at the University of Birmingham; **Miss G. M. Air,** to work at the MRC Laboratory of Molecular Biology, Cambridge; **M. J. Clemens,** to work at the National Institute for Medical Research, Mill Hill.

Professor Robert A. Good, Regent's professor of pediatrics and microbiology at the University of Minnesota, is to receive the **Howard Taylor Ricketts award** for 1970.

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Vacant: From October 1970 for 1 year, fully furnished 3-bedroom house in North London. Close to Northern Line (Whetstone–Totteridge station) with easy access to city centre. Central heating, garage and garden. Prefer **exchange** with similar in Boston–Cambridge, but will consider rental. Please contact Dr C. Hughes, National Institute for Medical Research, London NW7.

Wanted: 4-bedroom furnished house or apartment convenient for commuting to University College, London, from mid-September 1970 to July/August 1971. Could **exchange** for use of 5-bedroom house in Seattle, near University of Washington. Please contact Professor M. M. David, Chemical Engineering Department, University of Washington, Seattle, Washington 98105, USA.

Wanted: From January 1971 to December 1971, furnished house or apartment, 3 bedrooms with central heating, either within walking distance of University College, or on west or south-west side of London with good transportation, for professor, wife and three children, ages 9, 7 and 4. Please contact Dr Harold J. Bright, Department of Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.

Wanted: Furnished London home within 30 min of WC1 for 12 months starting September 1, 1970, for professor with young family. Central heating, 2–3 bedrooms, less than £65 monthly. Please contact Dr A. Kalantar, Chemistry Department, University of Alberta, Edmonton, Canada.

British Diary

Monday, June 1

Immunological Tolerance: Its Possible Role in Organ Transplantation (5 p.m.) Professor L. Brent, University of London, at the Wright-Fleming Institute, St. Mary's Hospital Medical School, Norfolk Place, London W2.

Tuesday, June 2

Magnetism (5.30 p.m.) Sir Lawrence Bragg, CH, FRS, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Fourth Form pupils from Schools in London and the Home Counties. To be repeated on June 3, 9 and 10.)

Management and Automation in the Petroleum Industry (four-day conference) Institution of Electrical Engineers, in association with the Institute of Petroleum, the I.Mech.E., the I.Chem.E., and the Inst.M.C., at Bath, Somerset.

Summer Meeting (five-days) Institution of Electrical Engineers, at Bristol.

Wednesday, June 3

Rotational Fabrics in Syntectonic Crystals, Dr D. W. Powell and Dr J. E. Treagus (demonstration); **Exhibit of Geological Map and Sections of the Kyrenia Range, Cyprus,** Professor W. D. Gill (demonstration); **The Structure of the Gulf of Suez (Clysmic) Rift,** with special reference to the Eastern Side, Dr D. A. Robson (paper, 5 p.m.) Geological Society, at Burlington House, Piccadilly, London W1.

Thursday, June 4

Cryosurgery in Surgical Practice (5.15 p.m.) Professor Sir James Fraser, University of London, in the Page Street Lecture Theatre, Westminster Medical School, Horseferry Road, London SW1.

Numerical Analysis of Partial Differential Equations (9.45 a.m., two-day discussion meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Friday, June 5

Clinico-Pathological Correlation in Biliary Tract Disease (11 a.m.) Mr N. Keddie, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

Saturday, June 6

Aspects of Bone Disease (10 a.m. symposium) British Institute of Radiology, at Queen Elizabeth Hospital, Edgbaston, Birmingham 15.

Reports and Publications

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Sight Without Glasses: an Explanation of the Working of the Visual System. By Simon Dale. Pp. 28. (London: Johnson Publications, 1970.) *10s 6d (53p)*. [173

University of Reading. Proceedings of the University 1968–69. Pp. 246. (Reading: The University, 1970.) [183

Flora of Tropical East Africa. Edited by E. Milne-Redhead and R. M. Polhill. Gramineae (Part 1). By W. D. Clayton. Pp. 176. (London: Crown Agents for Oversea Governments and Administrations, 1970. Obtainable from HMSO.) 21*s*. [183

Bulletin of the British Museum (Natural History). Zoology. Vol. 19, No. 3: Skull and Swimbladder Connections in Fishes of the Family Megalopidae. By P. H. Greenwood. Pp. 119–135 + 3 plates. (London: British Museum (Natural History), 1970.) 20*s*. [183

Evidence to the Roskill Commission—Some Regional Factors Affecting the Choice of a Site for London's Third Airport. By Ray Thomas and Anne Whalley. Pp. 21. (London: Political and Economic Planning, 1970.) [193

Metropolitan Water Board. Forty-third Report on the Results of the Bacteriological, Chemical and Biological Examination of the London Waters for the years 1967–1968. By Dr E. Windle Taylor. Pp. 130. (London: Metropolitan Water Board, 1970.) 32*s* 6*d*. [193

Integrated Circuit Devices. (Aylesbury: SGS (United Kingdom), Ltd., 1970.) 21*s*. [203

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 266, No. 1174 (19 March, 1970): The Free Oscillations of Fluid on a Hemisphere Bounded by Meridians of Longitude. By M. S. Longuet-Higgins and G. S. Pond. Pp. 193–223. (London: Royal Society, 1970.) 17*s*; $2.20. [233

Natural Environment Research Council. Annual Report of the National Institute of Oceanography for the years 1966–67 and 1967–68. Pp. 95. (Wormley, Godalming: National Institute of Oceanography, 1970.) 8*s* 6*d*; $1.25. [233

The Macaulay Institute for Soil Research. 1968/1969 Annual Report, No. 39. Pp. 68. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1970.) [233

The Public Schools Commission. Second Report, Vol. 1: Report on Independent Day Schools and Direct Grant Grammar Schools. Pp. xv + 183. (London: HMSO, 1970.) 18*s* (90*p*) net. [243

Infra-red Astronomy. By Professor James Ring. (Inaugural Lecture, 4 June 1968.) Pp. 199–211. (London: Imperial College of Science and Technology, 1970.) 7*s*. [243

The Accountant, The Computer, and the Abacus. By Professor Trevor Gambling. (An Inaugural Lecture delivered in the University of Birmingham on 12th February, 1970.) Pp. 20. Eugene Ionesco. By Professor R. J. North. (An Inaugural Lecture delivered in the University of Birmingham on 13th November, 1969.) Pp. 16. (Birmingham: The University, 1970.) [253

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 258, No. 819 (26 March, 1970): The Late-Weichselian Flora of the Isle of Man. By Camilla A. Dickson, J. H. Dickson and G. F. Mitchell. Pp. 31–79 + plates 1 and 2. (London: The Royal Society, 1970.) 30*s*; $3.90. [263

Building Research Station Digest, No. 115: Soil and Waste Pipe Systems for Office Buildings. Pp. 8. (London: HMSO, 1970.) *9d.* [263

Gardens to Visit 1970. Pp. 64. (Dorking, Surrey: The Gardeners' Sunday Organization, c/o Mrs K. Collett, White Witches, Claygate Road, 1970.) *1s 6d.* [313

Gelatine and Glue Research Association. 21st Annual Report, October 1968–September 1969. Pp. 61. (London: The Gelatine and Glue Research Association, 1970.) [313

MS30 Double Beam Mass Spectrometers. Pp. 28. (Urmston, Manchester: AEI Scientific Apparatus, Ltd., 1970.) [313

Library Association Research Publications. No. 1: Classification and Information Control. (Papers representing the work of the Classification Research Group during 1960–1968.) Pp. 130. *30s* (L.A. members *24s*). No. 2: Classification for a General Index Language: A Review of Recent Research by the Classification Research Group. By D. J. Foskett. Pp. 48. *10s* (L.A. members *8s*). (London: The Library Association, 1969 and 1970.) [14

Alcohol Abuse. Pp. 40. (London: Office of Health Economics, 1970.) *3s.* [24

Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme. Sixteenth Annual Report. Pp. 13. (London: Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme, 1970.) [24

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Vol. XXV, No. 9: The Movements of Oystercatchers *Haematopus ostralegus* L. Visiting or Breeding in the British Isles. By P. J. Dare. Pp. v + 137. (London: HMSO, 1970.) *50s* (£2.50) net. [24

Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research. 1969 Report. Pp. 71. (London: Ciba Foundation, 1970.) [34

Fabian Research Series, No. 286: Action for Welfare Rights. By David Bull. Pp. 24. (London: Fabian Society, 1970.) *3s* (*15p.*) [34

Society for Research into Higher Education, Ltd. SRHE Working Party on Teaching Methods—Publication 2: Aims and Techniques of Group Teaching. By M. L. J. Abercrombie. Pp. 32. (London: Society for Research into Higher Education, Ltd., 1970.) *9s* (members *6s*). [34

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Soil Publication No. 26: Growth Rates of *Pinus Radiata* D. Don in Relation to Foliar Levels of Nitrogen and Phosphorus for Plantations in the South-East of South Australia. By M. Raupach, R. Boardman and A. R. P. Clarke. Pp. 28. (Melbourne: CSIRO, 1969.) [103

US Department of the Interior: Geological Survey. Water-Supply Paper 1920: Surface Water Supply of the United States 1961–65. Vol. 1: Lower Mississippi River Basin Except Arkansas River Basin. Pp. x + 1103 + 1 plate. (Washington, DC: Government Printing Office, 1969.) [113

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Department of Applied Microbiology

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(Reference A.M.39)

or

SENIOR TECHNICIAN

(Reference A.M.40)

Applicants must have experience in Microbiological and/or Biochemical techniques. Preference will be given to holders of a suitable degree or H.N.C. qualification or its equivalent. Duties are concerned with industrial contract work, in the Centre for Industrial Innovation, under the Department of Applied Microbiology.

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(Reference A.M.41)

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Applications in writing, quoting reference numbers, giving details of age, experience and qualifications, to Mr. G. M. Boyd, Finance Office, University of Strathclyde, George Street, Glasgow, C.1.

(2106)

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DEPARTMENT OF BIOLOGY

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Applications, including details of experience and addresses of two referees, should be sent to the Bursar, The University of York, Heslington, York, not later than June 30, 1970. (2112)

TECHNICAL INFORMATION

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Pfizer

D. W. Sells, Personnel Manager,
Research Division,
Pfizer Limited,
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(2158)

TRINIDAD & TOBAGO COCONUT RESEARCH LTD.

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Applicants should hold a degree in Agriculture or in the Natural Sciences and should have had a number of years' experience in Field Experimentation.

Applications and requests for further information should be addressed to:

Trinidad & Tobago Coconut Research Ltd.,
P.O. Box 295,
Port of Spain, Trinidad, W.I.

(2157)

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P. G. T. Butler, Personnel Officer,
Prochem, Special Gasses,
Deer Park Road,
London SW19.
Tel: 01-542 6677.

(2189)

Huntingdon Research Centre

Research officer or assistant to work on quantitation of lung and skin irritation and the development of bioassays of irritant effects. The laboratory is equipped with the latest instrumentation for image analysis in pathology and carries out active research in this field. The post could suit either a research worker qualified to B.Sc. standard or an experienced technician holding FIMLT, AIMLT or HNC qualifications. Excellent opportunities for publications or thesis. The post is a responsible one and salary will not be less than £1,500 per annum.

Application to: **C. Urwin,**
Division of Pathology,
Huntingdon Research Centre,
Huntingdon.

(2135)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF ANATOMY

Applications are invited from registered Medical or Science graduates, male or female for the post of LECTURER IN ANATOMY AND HISTOLOGY. The appointment carries responsibility for teaching preclinical medical students at ordinary and honours levels. Previous teaching experience is an advantage. The appointee must engage in research work and candidates with strong investigative inclinations will be favourably considered. Duties to commence October 1, 1970. Salary will be within the scale £1,355 by £125 to £3,105. Permanent appointment of medical graduates at not less than £1,585.

Applications (two copies), together with the names of three referees, should be received by the Registrar, University College, Cardiff, from whom further particulars may be obtained not later than June 12, 1970. Please quote ADV/465/N.

(2110)

UNIVERSITY OF DUBLIN
Trinity College

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Applications are invited for the above post which has been established in conjunction with the Irish Flour Millers' Association.

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Further particulars may be obtained from:
The Assistant Secretary (Staff),
Trinity College,
Dublin 2.
who will receive completed applications up to June 22, 1970.

(2109)

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(UNIVERSITY OF LONDON)

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For further details write to Dr. D. N. Kirk, Chemistry Dept., Westfield College (N), Hampstead, N.W.3, to whom applications should be addressed before 30th June, 1970.

(2203)

PAPUA AND NEW GUINEA INSTITUTE OF TECHNOLOGY

(formerly Institute of Higher Technical Education)

Applications are invited for the following appointments in the School of Engineering:

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Further information about the Institute, method of application and conditions of appointment (including leave and passages, F.S.S.U. type superannuation, study leave, etc.) may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, WC1H OPF.

Applications close on June 26, 1970.

The Director of the Institute (W. E. Duncanson, Ph.D.(Camb.), D.Sc.(Lond.), F.Inst.P., F.I.E.(Aust.), C.Eng.) will be in the United Kingdom for consultation and interviews up to mid-July. Contact with him may be made through the Association's office. (2183)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BOTANY AND PLANT TECHNOLOGY

Applications are invited for the post of Lecturer in Plant Pathology. Preference will be given to those with special interest in the more experimental aspects of diseases of crop plants caused by bacteria and fungi.

Salary according to age and experience in scale £1,240 by £115 to £2,850 plus £60 per annum London Allowance (Salary and Allowance under review).

Applications, with a curriculum vitae and the names of two referees, should be sent to Professor C. P. Whittingham, Imperial College, London, S.W.7, to arrive not later than June 15. (2159)

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FISONS

(2126)

Plant Pathology Laboratory

CHEMIST

The Laboratory, which is part of the Ministry of Agriculture, Fisheries and Food, and located at HARPENDEN, HERTS., needs a Chemist (graded Experimental Officer/Assistant Experimental Officer) to join an expanding group concerned with plant and soil analysis for pesticide residues. Techniques used in this work include gas, paper, column and thin layer chromatography, spectroscopy and polarography. The group is investigating problems arising from the commercial use of insecticides, fungicides and herbicides and is also involved in the development of analytical methods for these compounds.

Salary: £1,020 (at 22)–£1,311 (at 26 or over)–£1,578; EO £1,725–£2,177.

Qualifications: Degree, HNC, or equivalent in appropriate subject. Age: AEO under 28; EO normally 26–30.

Prospects of permanent pensionable appointment. Prospects of promotion.

APPLICATION FORMS from the Director, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts. **Closing date: 15 June 1970.**

(2123)

SCIENTIFIC CIVIL SERVICE

FISHERIES LABORATORY,
Ministry of Agriculture,
Fisheries and Food,
Burnham-on-Crouch, Essex

CHEMIST (graded Experimental Officer) required to develop a system of monitoring the occurrence and distribution of important polluting substances such as pesticide residues and heavy metals in the seas around Britain. The materials to be sampled and analysed may include fish, shellfish and other invertebrates, plankton, seawater and seabed materials.

The successful candidate will assist in the planning and organisation of an extended sampling system and take direct day-to-day responsibility for analysis within the laboratory. Duties may involve seagoing in the Ministry's research ships or in commercial fishing vessels.

Qualifications: See below. Extensive working knowledge of modern methods of chemical analysis and several years of practical experience in analytical work essential.

A starting salary above the minimum of the scale may be recommended.

Non-contributory pension. Prospects of promotion.

APPLICATION FORMS are obtainable by writing to the Civil Service Commission, 23 Savile Row, London W1X 2AA, or by telephoning 01-734 6010 ext. 229 (after 5.30 p.m. 01-734 6464 "Ansafone" service). **Please quote S/94-95/70/MAF/42/G. Closing date: 22 June 1970.**

FISHERIES LABORATORY,
Ministry of Agriculture,
Fisheries and Food,
Burnham-on-Crouch, Essex

BACTERIOLOGIST (graded Experimental Officer) to join a team engaged in marine pollution studies with particular interest in the effect of pollution on shellfish and inshore fisheries. The work is concerned with field investigations at many points around the coasts of England and Wales. Current projects include: adaptation of existing methods of mussel and oyster purification to new situations; studies of the multiplication of bacteria in stored shellfish; sanitary surveys of shellfish beds; the transmission of viruses and the heat sterilization of shellfish. The publication of results will be encouraged.

Qualifications: See below. Candidates should have extensive practical experience and wide competence in microbiological techniques, whilst a knowledge of methods of water examination is desirable.

Non-contributory pension. Prospects of promotion.

APPLICATION FORMS are obtainable by writing to the Secretary, Civil Service Commission, 23 Savile Row London W1X 2AA, or by telephoning 01-734 6010 ext. 229 (after 5.30 p.m. 01-734 6464 "Ansafone" service). **Please quote S/95/MAF/29/G. Closing date: 22 June 1970.**

MARINE LABORATORY,
Department of Agriculture and
Fisheries for Scotland,
Aberdeen

Three posts for **CHEMIST/BIOCHEMISTS and BIOLOGIST** (graded Experimental Officer/Assistant Experimental Officer).

Post 1. Chemist/Biochemist to work as member of a team engaged on the investigation of the biochemical and physiological effects of pesticides and related organochlorine compounds in fish.

Post 2. Chemist/Biochemist to work on analysis of micro-constituents in sea water and in marine organisms in a group studying the effects of pollution and marine food chains.

Post 3. Biologist to work in Demersal Fish Section on experimental work at sea and in aquaria, concerning growth and feeding habits of fish. Duties will include periods at sea and early morning attendance at the Fish Market to participate in the Laboratory's fish sampling programme. Men only should apply for this post.

Qualifications: See below. For post 3, experience of keeping fish in an aquaria would be an advantage.

Prospects of permanent pensionable appointments.

Prospects of promotion.

APPLICATION FORMS from Establishment Division, Room 171, St. Andrew's House, Edinburgh EH1 3DE. **Closing date: 15 June 1970.**

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(2200)

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The General Manager,
Associated Portland Cement Manufacturer Ltd.,
Research Laboratories, Greenhithe, Kent.

(2160)

UNIVERSITY OF EAST AFRICA
UNIVERSITY COLLEGE—DAR ES SALAAM

Applications are invited for the following appointments in the FACULTY OF AGRICULTURE:

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PROFESSOR OF AGRICULTURAL CHEMISTRY
LECTURER IN FOOD TECHNOLOGY
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LECTURER IN POLITICAL ECONOMY
LECTURER IN PEDOLOGY
FARM MANAGER (Senior Lecturer/Lecturer level)

Salary scales: Professor £EA3,150 per annum; Senior Lecturer, £EA1,950 to £EA2,615 per annum; Lecturer, £EA1,350 to £EA2,230 per annum (£EA = £1 3s. 4d. sterling). Salaries supplemented in range £612 to £1,068 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate cases by British Government. F.S.S.U. Family passages; biennial overseas leave.

Detailed applications (six copies), naming three referees by June 26, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (2121)

A RESEARCH ASSISTANT REQUIRED TO study the movement of soil solutes in the root zone of soils. Applicants should have an interest in applying physico-chemical techniques to problems in soil and plant nutrition. Familiarity with electronics, computer programming and the use of radioisotopes should be an advantage. Salary in the range of £1,000 to £2,000 depending on experience.—Applications, in writing, should be sent by June 15 to the Reader in Soil Science, Department of Agricultural Science, Parks Road, Oxford, who will answer all inquiries. (2139)

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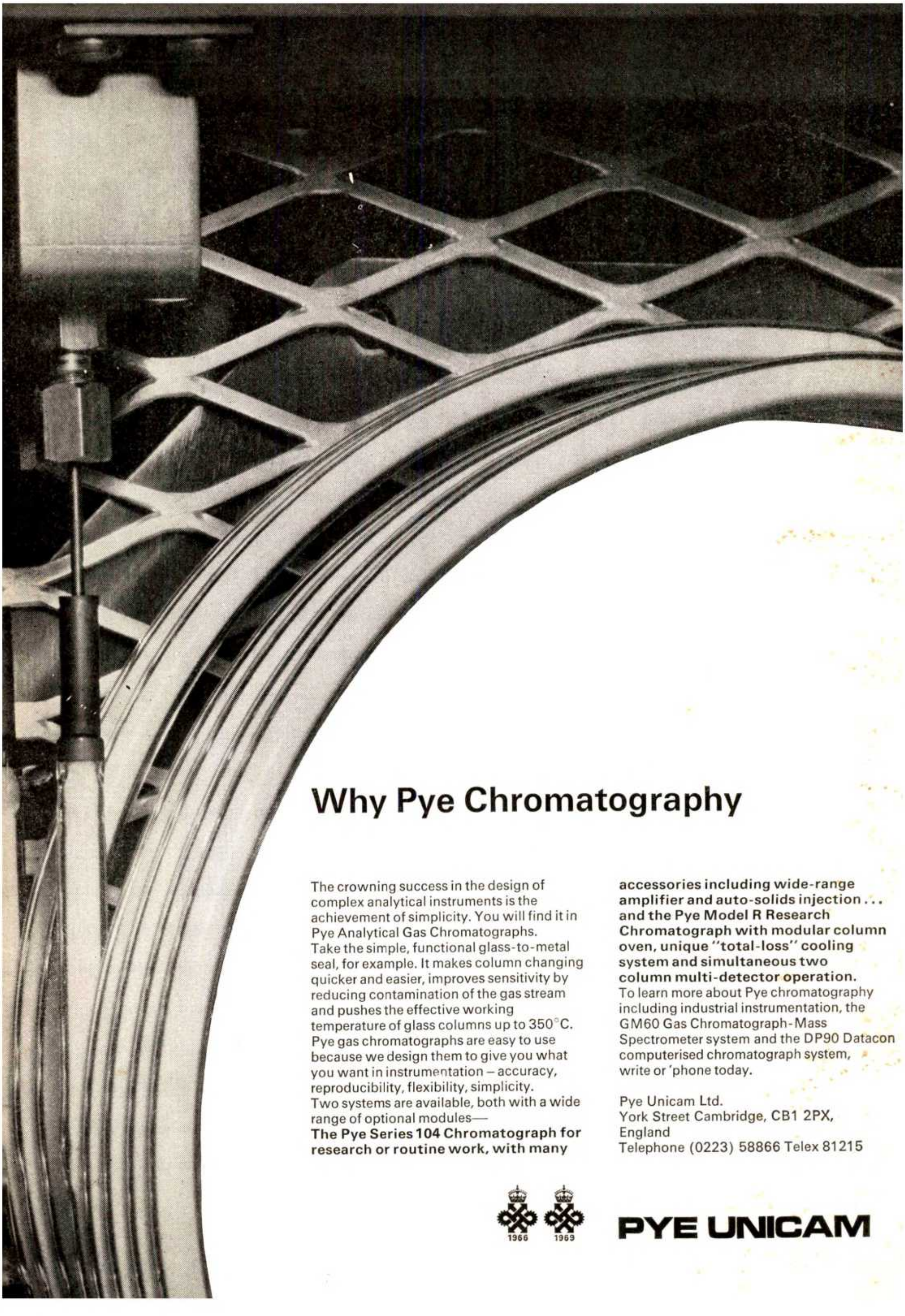

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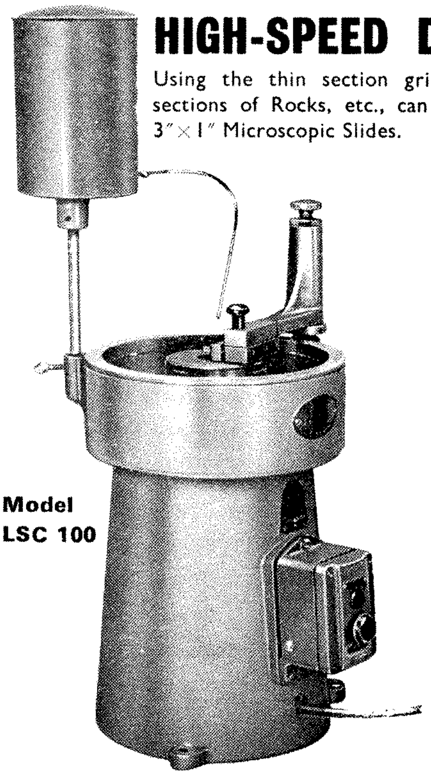

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NATURE Volume 226
JUNE 6, 1970

Who Should Count the Cost?

Mr Emilio Q. Daddario, the vigorous chairman of the House of Representatives Subcommittee on Science, Research and Development, has a great many friends and the best among them should tell him that he is barking up the wrong tree in trying to foist on the rest of Congress what he and Mr Charles A. Mosher call the Office of Technology Assessment (see page 894). The chief difficulty, all too apparent in the past few months, is that the doctrine of technology assessment has become one of those which are generously applauded even when their meaning is obscure. Briefly, the phrase is meant to evoke all kinds of perfectly proper sentiments about the need to make sure that the consequences of technical innovations are predicted as carefully as possible, preferably by making some attempt at a cost-benefit analysis. It first gained wide currency a year ago, when separate reports appeared in the United States by committees of the national academies of science and of engineering urging the need for technology assessment in language frequently so obscure that only the good intentions of the authors were permanently fixed in public consciousness. More recently, in November and December last year, Mr Daddario's subcommittee held a public inquiry on the subject which produced a long list of cautionary tales about the care which must be exercised in bringing new devices into service in the modern world, but which also saw the first signs of a movement now enshrined in the bill which Representatives Daddario and Mosher have introduced to provide Congress with an agency of its own specializing in technology assessment. Nobody will question their intentions, but there must be the gravest doubt about the feasibility of what they now propose.

The proposal for a congressional agency to help with making assessments of the value and of the consequences of technical development stems in part at least from the sense which must oppress a good many members of the Senate and the House of Representatives that the Administration is so well provided with technical assistance of all kinds that it can make rings round Congress on matters as diverse as the safety (or otherwise) of DDT, the advantages and the snags of supersonic transports and the sufficiency of particular programmes for the relief of water pollution. Is there not, in other words, a need that Congress should be better equipped to answer back? In terms like these, it is hard to deny the message in the bill. The question that should be asked, however, is whether the particular device which is now being advocated is the most suitable for its stated purpose. The question is of more general importance than its strictly American origins would suggest, for all kinds of legislative bodies in advanced societies find themselves increasingly at a loss to know how to make rational judgments on increasingly complicated technical questions.

The practical objection to Mr Daddario's proposal for an Office of Technology Assessment is that such an organization would have to be staffed by men of outstanding and almost unprecedented intellectual strength and moral virtue if it were to carry weight with the congressional committees which are most in need of the help which Mr Daddario is anxious to provide. The problem is well illustrated by the issue of the supersonic transport, also a live issue in Washington last week (see page 896). For several weeks, Senator Proxmire's subcommittee has been chivvying away at a score of witnesses representing as many different interests in the hope of forming a recognizable view of the importance and the disadvantages of the project. The fact that Senator Proxmire would probably be happiest if his inquiry sustains a case against the supersonic transport is probably irrelevant. The interest of the process has been the way in which it has uncovered disagreements within the government about the virtues of the project—Mr Russell Train, the chairman of the Environmental Council, turned out to be a surprisingly strong opponent. No amount of academic study by an Office of Technology Assessment could have uncovered this kind of internal conflict. To be sure, if the office had produced a formal report on the subject of supersonic flights, it would have been possible for congressional inquiries to proceed exactly as they do at present, but congressional committees would then find themselves in the unwelcome role of having to defend opinions of their own and not to challenge the intentions of the Administration. It is unthinkable that Congress would change its spots in such a casual fashion. The truth, of course, is that even matters of technology assessment are political issues in the broadest sense—settling the issue of the supersonic transport will require that somebody should balance the interests of those who want to have supersonic aircraft against the interests of those who wish that they will never fly. To the extent that the proposal for an Office of Technology Assessment would carry matters like these outside the realm of politics, it is unrealistic. The chances are that most people in Congress would regard it as such.

But if the Office of Technology Assessment is not the right instrument, what methods should be used for helping Congress to form a more balanced judgment about the advantages and disadvantages of innovation? To the extent that the task of Congress is to keep a sceptical eye on what the Administration is doing with public money, one of the most difficult tasks is to identify the problems where there is need to worry. A little thought will show that in many of the situations in which in recent months it has been necessary to put a stop to some accepted practice—the unrestricted use of DDT, for example, or the discharge of raw sewage into rivers—there has been no obvious occasion when Congress would have had natural cause to intervene at a somewhat earlier stage. The licensing of pesticides is, after all, a comparatively recent practice. Rivers have been regarded as open sewers for centuries. Moreover, the attempts—successful as they have been—to provide more rigorous public controls on practices like these have sprung from the proper activity of independent pressure groups bringing their case to Congress. In the clamour to be heard, those with sober warnings to give have been identified by the good sense of their case. If Congress really wishes to improve the degree to which society can control the uses made of new technology, the best thing will be to stimulate and to encourage the organizations which are independent both of the Administration and the legislature to make reasoned cases for or against particular innovations. Rather than setting up an entirely new organization to make academic studies of these matters, Congress would be better advised to be even more ready than at present to listen to what outsiders have to say.

But who is to provide not merely encouragement but money? The trouble, of course, is that making careful studies of the advantages of some technical innovation is a slow and expensive business. Even governments are not sufficiently contemplative and well provided with good technical advice to come to sensible decisions—a point well illustrated by the way in which the present Administration in the United States has banned the use of detergents containing phosphates from the beginning of next year without knowing whether the approved alternative (called NTA) may be even more damaging when it finds its way into sewage treatment plants. In circumstances like these, the best procedure would be to find some easily acceptable means by which independent studies of technical innovations could be financed by public money after having been approved by Congress. But is this not one of the ways in which the National Science Foundation could put some welcome flesh on the bones of its intention to spend more money on work which has some social relevance? What, in particular, is to prevent the National Science Foundation from spending money on studies intended to help with problems which worry Congress? The fact that it would often be necessary to go outside the universities proper to find suitable advisers is not to be scoffed at but rather welcomed.

Naming Mountains after People

One of the more informal means of patronage in the United States, and one of the most confusing, is the way in which mountains in Antarctica are named after living people, many of whom are distinguished administrators of science. Last week, on the twentieth anniversary of the setting up of the National Science Foundation, Dr Lee DuBridge, Scientific Adviser to the President, was told that the Board on Geographic Names had decided that a range of mountains in northern Victoria Land would in future be known as DuBridge Range. On the same occasion, Dr Philip Handler, who gave up the chairmanship of the National Science Board last week, was told that another inhospitable tract of Victoria Land, a ridge rising to more than 8,000 feet in what are called the Victory Mountains, will in future be known as Handler Ridge. Nobody expects that the two people whose names are thus made geographically permanent will ever climb the heights named after them. Indeed, it is no disrespect to either of them to wonder whether they will ever clap eyes on these outlandish places. No doubt they will have some splendid photographs to hang above the mantelpiece—the National Science Board took care that one of its members flew over the region in December—but that is likely to be the limit of their acquaintance with these places.

Several questions come to mind. Although the recognition of distinguished public service by the naming of a mountain may be less pernicious than the equivalent British practice of turning ordinary mortals into knights—Dr Handler is unlikely to find that people defer to him now that an uncared for part of Antarctica is named for him—the practice is an awkward break with the tradition that place names on the surface of the Earth should say something about those who have discovered them or, alternatively, should be named for some other characteristic—shape, position or function. To be sure, in the naming of geographical places, there has also been a tendency to honour patrons—the Renaissance explorers were keen on this and Antarctica itself already has several names with such an origin. But is it now intended that Dr

DuBridge and Dr Handler should be commemorated on the map as scientists or as patrons of science? And if the latter, should not the chosen names be those of presidents or politicians, not public servants who merely spend the money which the politicians have the ingenuity to raise? If, on the other hand, they are commemorated as scientists, is there not now likely to be a clamour for a much more widespread naming of places? After all, there are a great many glaciologists, to mention only one small group, with something of a right to have their names scattered on the ice. But if, as is more probable, the present practice is merely a piece of sentiment of the kind which in itself does little harm, is there not a case at this time, when scientific research in the United States is hard pressed for lack of funds, for saying that place names should not be given away, but sold? Rumour has it that in circumstances when the Liberal Party in Britain was short of money, the then Prime Minister, Lloyd George, made a substantial sum by selling knighthoods. Would it not be much more to the point if the Board on Geographic Names could be persuaded to sell off names in Antarctica to people willing to put up some fixed sum for scientific research? With luck, they could hope for $100,000 or so for every mountain peak and perhaps even $1 million for something as elaborate as a ridge or range. But if the sale were conducted by public auction, it would be a great public service to see who bids and for what.

---

100 Years Ago

NATURE

Fleas may live as long as nine months. Scarcely any observations have been made on the length of life in the molluscoidea and mollusca. Fish appear to have great tenacity of life. Thus, the carp is believed to have attained the age of 150 years, and the pike 267 years, if a ring with the following inscription is genuine—"I am the fish which was first of all put into this lake by the hands of the Governor of the Universe, Frederick II., the 5th of October, 1230." It weighed 350lbs., and was 19 feet long. Its skeleton was exhibited at Mannheim, and it was taken at Halibrun in Suabia in 1497. Of the Amphibia, the toad lives 36 years, the frog 12 to 16, and various tortoises are inferred to be of great age from their size. Amongst birds, the parrot, goose, falcon, and raven are long-lived, the two former reaching 100 to 120 years, and the two latter exceeding 150 years. Wrens only live two or three years. Amongst mammals, the whale and elephant have the longest term of life, both probably exceeding 100 years, and possibly reaching 200; horse 25, but occasionally reaching 40 years; ox 15 to 20 years; sheep and goat 12 years; lion 20 to 50 years; cat 9 to 18 years.

From Nature, **2**, **99**, *June* **9**, **1870.**

OLD WORLD

SOVIET UNION

Lysenko Rides Again

by our Soviet Correspondent

ZHOREZ MEDVEDEV, author of the history of the Lysenko period and until recently the director of the Laboratory of Molecular Radiobiology at Obninsk, has been arrested and detained in a mental hospital. This has emerged from protests signed by a particularly eminent group of Soviet scientists which includes Academician Andrei Sakharov, the nuclear physicist whose second critique of the Soviet system was published two months ago, the physicists Petr Kapitsa and Valentin Turchin, the biochemist Vladimir Engelgardt, the biologist Vladimir Astaurov and Aleksandr Tvardovskii, the recently dismissed editor of Russia's leading literary monthly, *Novyi Mir*.

Zhorez A. Medvedev.

Yet Dr Medvedev is at least as significant for Soviet science as those who have bombarded the authorities with telegrams on his behalf. When, on Friday, May 29, Medvedev was arrested at his home in Obninsk and removed to a mental hospital in Kaluga, some 120 miles from the capital, his twin brother, Roi Medvedev, stated that this was due to Zhorez having openly protested against the "senseless barriers" imposed by the Soviet authorities between communication and cooperation among Soviet scientists and their colleagues abroad, and against the system of postal censorship imposed by those authorities.

Roi Medvedev neglects to mention his brother's chief offence in the eyes of the government, which must surely outweigh any bootleg protest literature intended for internal consumption—the fact that Zhorez Medvedev is the author of the outspoken exposé of Soviet genetic policy under the Stalin regime, *The Rise and Fall of T. D. Lysenko*, which, in spite of the official retreat from Lysenkoist genetics and Stalinist policies during the mid-60s, could not appear in Russia because it is too critical of the regime, but which was published last year in English translation by the Columbia University Press.

The picture which Medvedev presents is one of scientific theories "tailored" to fit in with official philosophies and attitudes (the molecular theory of heredity was equated to Nazi racism, and hence not only condemned by academicians, but built up into a scientific bogeyman in the propaganda press), the supporting of the official theories of vernalization and nutrient-conservation by falsified evidence, false claims, or simple fiats in the form of telegrams from Lysenko to high-ranking officials.

Perhaps even more alarming than the excesses of Lysenkoism is Medvedev's answer to the question of how these happened. False doctrines, says Medvedev, are natural to science, they are "the extreme variants of essential hypotheses, assumptions and theories", which under the natural conditions of scientific progress will be eliminated by further experiments and by the free discussion and interchange of ideas with other researchers in the field or in allied fields. They can achieve "a monopolistic position only in state systems that are extremist in nature, as a particular manifestation of many other deviations from the norms of organized human society".

Shortly after the appearance of his book abroad, Medvedev was dismissed from his post as director of the Laboratory of Molecular Radiobiology at the Institute of Medical Radiobiology in Obninsk. His present confinement in the mental hospital will prevent him from attending an international conference on genetics. The telegrams of protest resulted in the dispatch to Kaluga from Moscow of a special psychiatric commission, which pronounced him normal, but recommended that he be kept in hospital for a further week—apparently as a face-saving measure. Clearly, Medvedev is seen as an opponent, not only of the Stalinist personality cult and the excesses which it produced, but of present policies also. It is noteworthy, perhaps, that another protest organized by Academician Sakharov denounced the holding in a mental hospital of Major-General Petr Grigorenko, who wrote a clandestine article in favour of the now-banned work of the historian Aleksandr Nekrich, *June 22, 1941*, an indictment of Stalin for ignoring the Nazi threat to Russia and by weakening the defence capacity of the Soviet Union. The rehabilitation of Stalin has been in progress for some time now. The Medvedev affair could be a sign that Lysenko may be due for rehabilitation in his patron's wake.

INVENTIONS

Resolving a Patent Muddle

WITH patent offices around the world breaking down largely because of the pressure of patent applications from abroad, two agreements which could ease the load are being hammered out. In Washington the Patent Cooperation Treaty is being negotiated and could even be signed within three weeks. This would ease the pressure on patent offices by sharing the work of checking whether an invention has been anticipated, but the granting of patents would still be up to the national patent offices. A more radical scheme, but one which is limited to western Europe and is not so truly international, is being discussed by eighteen countries including Britain. This aims at the setting up of a European Patent Office and involves some decline in the status of national patent offices.

Although a draft convention that deals with the way patent applications would be treated under the European scheme has already been published, the establishment of the scheme seems to be four or five years away. An international patent system has been as elusive as the philosopher's stone for more than a century, and there are many obstacles in the way of a European agreement. For example, there is the question of what is patentable—in Italy pharmaceuticals are excluded—and the durations of patents differ from one country to another.

The eighteen countries involved in the European negotiations are the EEC and EFTA countries together with Greece, Ireland, Spain and Turkey. The scheme is to consider the mechanism of patenting in two parts: what happens before a patent is granted and what happens afterwards. After a year's intensive effort the first part of the process has been covered by a draft convention, which envisages that a European Patent Office would examine applications for novelty in particular, and would grant patents that would be effective in all the eighteen countries. The location of the European office is still the subject of bargaining, but The Hague and Munich are believed to be candidates, and the possibility of some applications being examined in branch offices, for example in London, is not ruled out.

Once a patent is granted the next step depends on whether or not the state is a member of the EEC. For the purposes of the European patent the EEC is treated as a single country and a uniform legal system, under which the European patent will have force throughout the EEC, will be established to deal with such matters as patent infringement.

A European Patent Office could find itself dealing with something like 40,000 applications a year, and despite the difficulty of estimating the running costs of an international organization it looks as if the system will lead to savings for anyone who wants to file a patent in three or more countries.

The diplomatic conference on the European agreement is unlikely to take place before 1971. In the meantime there is the Patent Cooperation Treaty being negotiated in Washington which involves a number of offices to which patent applications will be sent for a report on novelty. After eighteen months the application is then published together with a list of the documents where the invention has been anticipated. It is then up to the national patent office whether or not to grant a patent. There are likely to be searching offices in the United States, Germany, the Soviet Union, Japan, and in The Hague, where the International Patent Institute is sited, which will also be the searching authority for the European scheme.

Although in principle the national offices will be able to ease the load by delegating the search procedure through the treaty, it remains to be seen to what extent the national offices are prepared to do this. In the end economic necessity will be the driving force.

LUNAR SAMPLES

SRC asks for More Moon

by our Astronomy Correspondent

Applications by British scientists for samples from the next batches of Moon rock and dust are expected to be forwarded to NASA within the next few days, just as soon as the applications have received the stamp of approval of the Space Policy and Grants Committee of the Science Research Council. Last week the applications were vetted by the Lunar and Planetary Panel, the committee of the SRC under the chairmanship of Professor K. Runcorn that deals with the lunar samples programme. It is believed that proposals from twenty-five groups will be forwarded to NASA, including experiments suggested by groups which properly come under the Natural Environment Research Council, and by groups in government laboratories.

The SRC is of course the scientific agency in Britain with which NASA deals and through which all applications for lunar material have to be channelled. Fifteen teams in Britain were on the SRC list for receipt of samples from the Apollo 11 and Apollo 12 missions, so it seems that interest has widened considerably. But the SRC is not saying at this stage what new experiments are being proposed, or indeed who the experimenters are, although it appears that no out of the ordinary proposals have been put forward. It seems safe to assume that the samples will be subject to a similar battery of tests as before: mineralogical and petrological examination, elemental analysis, age determination, examination for organic chemicals, analysis of physical properties (notably magnetism and luminescence), and so on.

The applications are for samples of the material which will be returned by the remaining Moon missions, Apollos 14 to 19. But the Science Research Council believes that NASA will provide further opportunities to apply for samples as the Apollo programme develops.

Nevertheless, there is still concern among scientists in Britain that liaison between the SRC and NASA is not as good as it could be. Complaints that NASA is weak on giving advance notice of opportunities to participate in space experiments are common, although this seems to be a problem that also afflicts scientists in the United States. But with interest in lunar and planetary science booming in Britain, and the omens good for the proposed school of planetary science at Lancaster University, people are looking for closer contact with the American programme. One idea which has been put forward by NASA is for a British scientist nominated by the SRC to work at Houston taking an active part in the Manned Spacecraft Center. The SRC refuses to comment because the NASA offer is apparently unofficial at present. People in the planetary sciences were hoping that the SRC would jump at the chance.

POLLUTION

Booms Banned; Exhausts Exempted

Noise and smoke are the chief targets of the White Paper on the environment published by the British government on May 28. If the government is re-elected, commercial supersonic flights will not be allowed to make sonic booms overland, and existing aircraft may be required to be quieter in taking off and landing. Heavy road vehicles will have to be checked annually for noise, and all new vehicles will need to be fitted with a device to reduce emissions of unburned hydrocarbons. More industrial processes will come within the scope of the Alkali Act, which controls air pollution, and reluctant local authorities in badly polluted areas will be compelled to ban domestic coal fires. Together with proposals for cleaning up land and water, these plans for legislation would be put into action during the lifetime of the next government, the Secretary of State for Local Government and Regional Planning, Mr Anthony Crosland, said when he introduced the White Paper (*The Protection of the Environment*, HMSO, London, 15p).

By deciding to outlaw the sonic boom, the government has reduced the potential sales of the Concorde (although the British Aircraft Corporation has always assumed in its sales projections that Concorde will be banned from flying overland), and other countries beneath the transatlantic air routes from Europe have already indicated that they intend to ban supersonic overflying. Without a ban by Britain, Concorde might sell 80 per cent more than the 250 planes expected to be sold by 1980. But those who have been complaining that the Concorde project puts profits before people are heartened by the decision, and the Noise Abatement Society talks of the proposal to ban overland commercial flights as a "splendid achievement", even though supersonic test flights over Britain this summer are to continue.

The proposals for clean air have been greeted with more scepticism. The trouble is that next winter there will not be enough smokeless fuel to go round because the output of gas coke has fallen now that natural gas is widely used, and many smoke control orders are being suspended to allow people to heat their houses with coal. As a result, the White Paper admits that the clean air programme will have to be delayed. This does not please the director of the National Society for Clean Air, Rear-Admiral P. G. Sharp, who is not confident that government plans for keeping open a few uneconomic gas works will be enough to solve the fuel shortage quickly. He would like to see more encouragement now instead of later as the White Paper implies, for people to use electricity, gas or oil instead of solid fuels. He is also disappointed that there are no plans for reducing the carbon monoxide content of vehicle exhausts—Mr Crosland's view is that the British climate does not warrant such drastic measures as are necessary, for example, in Los Angeles, even though the Economic Commission for Europe has recommended a general reduction of carbon monoxide emissions.

On waste disposal and water pollution, the government will let itself be guided by some half-dozen official reports which are due to appear within the next year, but it promises eventually to undertake a national clean rivers programme and will, in any case, be restructuring the water and sewage industry when the pattern of local government is reorganized. The White Paper is more specific about the penalties that should be imposed for pollution offences, which at the moment are absurdly small (£100, for example, for illegal discharges into rivers). These fines, the White Paper says, should be "brought into line with the realities of modern life", and the recent Oil in

Navigable Waters Bill has already made a start by providing for an increase from £1,000 to £5,000 in the fine on summary conviction for the master of a ship which illegally discharges oil into the sea.

CS GAS

Infringing the Gas Laws

THE British government's decision to regard CS gas as being outside the scope of the Geneva Protocol on chemical and bacteriological weapons came under attack from several quarters at a meeting of the British Pugwash Group last week. It was unfortunate that the debate took place in something of a vacuum since the top billing for the meeting, Lord Chalfont, was prevented by the election from explaining the logic of the government's position and no understudy from the Foreign Office was prepared to stand in at short notice. Lord Chalfont's absence forced participants to speculate about the official reasons behind the decision, and most of them had considerable difficulty in finding any logical arguments to back it up.

Professor Andrew Martin, QC, professor of international law at the University of Southampton, argued that the decision cannot really be justified on the grounds that CS is, in Mr Stewart's words, "not significantly harmful, except in exceptional circumstances", because the Geneva Protocol itself makes no distinction between lethal and non-lethal weapons. The protocol prohibits the use in war of "asphyxiating, poisonous or other gases, and of all analogous liquids, materials or devices", and its interpretation hinges to some extent on the phrase "other gases"—particularly because the equally authentic French version uses the phrase "ou similaires". In 1930, the British government issued a memorandum in which it said that tear gases fall within the scope of the protocol, and last July U Thant, Secretary General of the UN, called upon member states to make a clear affirmation that the protocol applies to all chemical, bacteriological and biological agents. What, then, has induced the British government to reverse its former declaration and to ignore U Thant's plea ?

One of the more popular explanations canvassed at the meeting was that the government was considerably embarrassed by using CS to control its own citizens in Ulster, and at the same time giving an undertaking not to use it against an enemy in wartime. But in any case, there is nothing in the protocol which prohibits its use for riot control, and the government was not acting illegally. Moreover, France stated categorically last December that it regards CS as prohibited in warfare, in spite of the fact that it is often employed for riot control in the streets of Paris. Another explanation which seemed plausible to some was that the British government's decision was in part a gesture of solidarity with the US, which has never signed the Geneva Protocol and is using vast quantities of CS on the battlefield in Vietnam.

The most logical explanation for the government's decision is, however, that it believes that CS is more humane than bullets for overcoming an enemy. But even this explanation sounded less than convincing to most participants at the meeting, because it is invariably used in wartime in conjunction with conventional weapons, and is therefore often just a forerunner to bullets. In any case, the lack of published material about the toxicity of CS gave rise to fundamental disagreements among the speakers about the dangers of using the weapon. Professor R. B. Fisher, of the University of Edinburgh, for example, maintained that the concentration of CS needed to cause significant physiological damage is several orders of magnitude greater than the concentration that can be tolerated, and that it is therefore unlikely that anybody could be exposed for long enough to suffer serious harm, unless he were incapacitated. On the other hand, figures quoted by Dr Julian Perry Robinson and Dr G. R. N. Jones suggest that doses of CS which would cause serious damage and even death might be much lower than Professor Fisher's estimates. The effect of CS on the eyes is also rather uncertain.

In any case, as Professor Fisher pointed out, it is illogical to campaign against CS on its own. The most important issue is to get the government to state that all chemical agents, including tear gases, are covered by the Geneva Protocol.

ZOOS

Acknowledged to Exist

THE response of the British government to the management consultants who recommended financial aid for the Zoological Society has been to provide £1,350,000 towards rebuilding and payments of debts, and to waive repayment of a loan of £125,000 made to the Society in 1964. Mr John Silkin, Minister of Public Building and Works, acknowledged in the House of Commons last week that the zoo has become, in fact if not in form, a national institution which is an important London amenity and tourist attraction.

The management consultants were called in by Mr Silkin's predecessor, Mr Anthony Greenwood, who announced last year a short-term government loan of £375,000 for the Zoological Society. This was in recognition of the mounting financial difficulties of a society that had always remained independent of government subsidy. The society's spectacular success in raising £3·5 million during the past ten years had not been enough to avoid deficits of £30,000 in 1968 and £54,000 last year.

After examining the details of the society's affairs at London Zoo and Whipsnade Park, the consultants passed the organization with a clean bill of health, and concluded that financial stability could be achieved in the longer term with government help and the continuation of efforts to cut costs and increase revenue. The government was recommended to repay the short-term debts incurred by the society's extensive rebuilding programme, which reached the half way mark two years ago with the opening of a new small mammal house. A contribution to the cost of the remaining part of the rebuilding programme was also called for, and has been provided in the form of £700,000 to be spent during 1970–74. To repay the short-term debts, £650,000 will be provided from the Civil Contingencies Fund, and the government loan of £125,000 made to the society in 1964 will not now have to be repayed.

This money will complement the society's own efforts to make ends meet during the present time of trouble. Among the consultants' other proposals for increasing revenue was an increase in subscriptions from scientific fellows and associates, which is now under consideration. Benefactors, of course, continue to be generous; £125,000 was received recently for the building of a new primate house. The charge for admission to the London Zoo was increased in May from 7*s* 6*d* to 8*s* 6*d* for adults, and the question of further increases is under review.

The society has also been rigorous in making all possible economies, and at the annual general meeting on May 20 the secretary, Sir Solly Zuckerman, said that costs had miraculously been cut by 2 per cent during the past year, in spite of the many improvements that had been effected.

Sir Solly also said that the council of the society is hoping that by the end of the 1970s there will be an endowment fund to take care of all redevelopment needs. The Zoological Society evidently intends to continue to cherish its independence.

COMPUTER BOARD

Finney for Flowers

Next month Professor Sir Brian Flowers is to relinquish the post he has held for four years as chairman of the Computer Board, in favour of Professor D. J. Finney, University of Edinburgh. Professor Finney, who is director of the Agricultural Research Council Unit of Statistics, has been a member of the Computer Board since it was set up in 1966 and is expected to continue the board's policy of promoting regional computing centres around the country.

One of the first tasks facing the new board is to see how much of the original idea of regional computing centres can be salvaged. As Professor Flowers explained last year, there seems no way of preventing a university selected as the centre for a region from taking up an unfair share of the computer's time. It is one thing for the Computer Board to urge a university to provide facilities for other universities, he said, but it is another matter to make a centre truly regional.

Professor Finney is unlikely to lead his seven man board far away from the guiding lines laid down by the original Flowers report, which chose London and Manchester to be the major computing centres in Britain with Edinburgh as a special centre based on a multi-access system; this would be later followed by a further concentration of special facilities into regions. But the extent to which the board will have to modify this scheme, as intimated by Professor Flowers when he opened the new centre at King's College, London, last year, is likely to depend on whether the development of more powerful computers can keep pace with the rising demand for facilities. Another decision facing the board is whether or not to introduce a system of charging for university computing.

MATHEMATICS

'Q' and 'F' a Bar to Prodigies

The Joint Mathematical Council has joined the swelling ranks of those who are worried about the proposals for 'Q' and 'F' level examinations in the sixth form. In a statement which is being sent to the Schools Council, the JMC has expressed concern that the proposals will hold back the mathematically gifted child and will exacerbate the shortage of mathematics teachers. But the council is prepared to admit that the scheme holds advantages for the average child.

The basis of the JMC's case is that if the present subjects of pure and applied mathematics are condensed into a single subject, the specialist mathematician will suffer. Even if sixth formers were allowed to study mathematics and statistics up to 'Q' level, the statement points out, the specialist mathematician could only devote two-fifths of his time to mathematics during the first year in the sixth form, and only one third in the second year. The JMC believes that so drastic an alteration of the present arrangements, whereby a student can spend two-thirds of his sixth form studies on mathematics, will lower standards in schools and make it necessary to lengthen degree courses.

The JMC believes that the working party which put forward the 'Q' and 'F' level proposals paid too little attention to the numbers of teachers that would be required to carry out its suggestions. Some two to three hundred extra mathematics teachers would be needed, the JMC believes, if further mathematics were to be taught as a separate subject. The council is also worried lest the centralized examinations proposed with the 'Q' and 'F' levels should cause mathematics syllabuses to ossify, and it believes that teachers should have some say in the way that courses are structured.

A way to make sure that the gifted student will not suffer, the council says, is to allow some students to by-pass 'Q' level. This would help to reduce cramming for exams in the sixth form and would help to maintain the standards of 'F' level examinations.

HEALTH

Shutting Up Pandora

Like the Food and Agriculture Organization with its cheerful predictions of long-term food surpluses, the World Health Organization also has some good news to spread, chief of which is that the incidence of smallpox has fallen for the second year in succession to the lowest ever recorded. Another global eradication campaign, that against malaria, has now been extended to some four-fifths of the population in the world's originally malarious areas (*The Work of WHO 1969*. WHO, Geneva, 1970. 14*s*. $2.25).

Less success has been had with plague and cholera which claimed as many victims in 1969 as in the year before. And yellow fever was back in Africa for the first time in six years. In the northern hemisphere, the 1968–69 influenza season started earlier than usual and the A2 strain of the virus, which caused the large outbreak in Hong Kong in 1968, was responsible for numerous epidemics. The upward trend of venereal diseases continued in many countries, though being less marked for syphilis than gonorrhoea, despite the evidence of studies showing that two-thirds of the venereal infections treated by private doctors go unreported.

Parliament in Britain

Atomic Energy Bill

AMONG the legislation which failed to make the statute book before MPs left for their constituencies last week was the Atomic Energy Bill. The bill, which sought to remove the nuclear fuels business and the radioisotopes business from under the wing of the Atomic Energy Authority and to set up separate companies to take care of these activities (see *Nature*, **225**, 1089; 1970), had already been amended and passed by the House of Lords, and was awaiting final approval from the House of Commons. The next government—whatever its political persuasion—must put the bill through the whole parliamentary process for a second time before it can become law.

Defence Contracts

MR JAMES DICKENS asked more questions about contracts for defence research agreed between the universities and the Ministry of Technology. Dr Ernest A. Davies, Joint Parliamentary Secretary to the Ministry of Technology, told him that the ministry's total expenditure on such contracts in the aerospace field in 1968–69 was £1,170,000. The University of Southampton was the biggest single contractor, with contracts worth just over £130,000, the University of London had contracts worth £121,000 and contracts placed at the University of Manchester were worth £111,000. The research involved has both civil and defence applications, Dr Davies said. (Written answers, May 26.)

Research and Development

RESEARCH and development was the subject of several questions from Miss Mervyn Pike. She was told by Mr Edward Short, Secretary of State for Education and Science, that the most recent figures show that in 1961–62, 2·67 per cent of the gross national product was spent on research and development. This had increased to 2·77 per cent in 1966–67 and dropped to 2·73 per cent in 1967–68. Mr Short also said that the total current expenditure on research and development carried out in government departments is estimated to be £229·6 million in 1968–69. This compares with £168·7 million in 1964–65. Mr Anthony Wedgwood Benn, Minister of Technology, also told her that the net cost of running the Ministry of Technology's research establishments is about £21,700 per qualified scientist a year. This figure includes £12,000 in salaries for the scientist and his supporting staff, £6,900 for equipment and £2,800 for buildings. (Written answers, May 27 and May 28.)

Additional University Grants

SUPPLEMENTARY grants to universities, amounting to £20·703 million, were announced by Mr Edward Short in reply to a question from Mr Eddie Griffiths. They will cover the remainder of the quinquennium up to the first four months of the 1972–73 academic year, and are composed of a total of £16·818 million for recurrent expenditure and £3·885 million for capital expenditure. The chief reason for the extra grant is the recent increases in academic staff salaries, which are expected to cost about £8 million a year. (Written answer, May 28.)

Miscellaneous Intelligence

"DEVILS do not occur in cleared places, although they may be found in such areas if there are patches of scrub or high grasses, particularly if large populations inhabit the nearby bush. Devils live successfully in close contact with human settlement and activity, and field observations show that they are most numerous in coastal heath and sclerophyl forest. . . . Man is the principal predator of adult devils" (E. R. Guiler, *Australian Journal of Zoology*, **18**, 49, March 1970).

AFTER a slow start the opposing sides in the battle of the environment are warming up their rhetoric to what promises to be a long, hot summer. Last week the delegate of an American food manufacturing company was complaining to the Society of Chemical Industry in London that the people who want to see all additives banned from food—the organic gardeners, food faddists, and "ecology-minded hippies or econuts"—are often the same as those who would have marihuana made legal. The econuts also have the pesticide manufacturers running scared: "Our industry is frankly disturbed," Mr W. P. Evans, deputy chairman of the British Agrochemicals Association, said recently, "by the present tendency on the part of those who make their own assessments of isolated and imperfectly understood work and promote their individual views to the alarm of the general public. A situation is created in which without any facts or real understanding of the issues, a public pressure for hasty judgment and precipitate action is built up." Mr R. E. Ashworth, chairman of the same association, has also decided the time is right to don a martyr's clothing: "We cannot live in an environment where pesticides are a whipping boy and the cause to champion for every crank. . . . Semi-hysterical emotion is a pollutant that we cannot live with." What sort of emotions can Mr Ashworth have had in mind? Perhaps those of sentiments like that which appeared in a recent editorial in the *Journal of the Soil Association* (**16**, 76; 1970): "Pollution and contamination are the Hitler of today". Hitler versus the econuts: yes, there's no doubt about it, the atmosphere is being polluted.

LIKE those two simple variables on whose changing values couturiers make their fortunes, the hemline and the neckline, the estimates for Britain's population in the year 2000 plunge and rise wildly from year to year. Considering the frivolous way in which the government regards demography—in British universities the subject is chiefly supported by the Ford Foundation—it is not surprising that the government gets the kind of forecasts it deserves. In 1968, for example, it was fashionable to suppose, among the cognoscenti of the Registrar General's Office, that Britain's population of 54 million would add another 21 million to its numbers by the year 2000. Government planners who took any notice of this figure must have been badly caught out when the fashion veered last year to an increase of only 12 million between now and 2000. And last month Mr Crosland was telling a Commons committee that a figure of only 4 million is now the fancied increase for England and Wales (the increase for Britain as a whole, not yet released, is likely to be around 8 million). One thing is as plain as a pikestaff—any talk of a population policy is so much hot air until the government gets itself some decent crystal balls.

NEW WORLD

Scientists against ABM, MIRV, etc.

THREE out of the four ex-Scientific Advisers to Presidents of the United States appeared last week before Senator Albert Gore's Subcommittee on Arms Control, International Law and Organization to complain about the installation of antiballistic missile systems in the United States and about the plan to install multiple warheads on Minuteman missiles from a point later this summer. Although Dr G. B. Kistiakowsky, Dr Jerome B. Wiesner and Dr Donald Hornig have already made their opposition to these developments public, the occasion was outstanding for the eloquence with which they put their case. Most of what they said was also entirely acceptable to the subcommittee except that Senator Gore took less than kindly to Dr Wiesner's suggestion that the subcommittee should put back into its title the word disarmament which had been there until quite recently.

All three speakers were anxious to insist that the SALT talks in Vienna could be jeopardized by a mishandling of the ABM and the MIRV. Dr Hornig, Scientific Adviser to President Johnson, and the incumbent at the White House in 1967 when Mr Robert McNamara argued the case for a limited ABM system with the name Sentinel (not replaced by Safeguard), said last week that "this futile pursuit of actions" tends only to increase the risks of the modern world. "Anyone who surveys the course of the build-up of nuclear armaments in the last 25 years, whether or not it is characterized as a race, can only be struck by the insanity of the result." Like the other speakers at the inquiry, Dr Hornig complained that the United States seems unable to be satisfied with a modest superiority in weapons—the three or four to one advantage over the Soviet Union in the middle 1960s did not bring stability but, rather, an effort by the Soviet Union to install more long range missiles—SS 9, SS 11 and SS 13. One difficulty, he said, was that the arithmetic of superiority is more complicated than mere numbers would suggest. He pointed out that in the past few weeks, Mr Melvin Laird, the Secretary of Defense, has justified new expenditures on weapons by the United States by saying that "our concern is not about today or even next year . . . but about what the future may bring". The only hope, Dr Hornig said, of avoiding continued escalation is if the SALT talks turn out to be successful.

On the Safeguard missile, Dr Hornig argued first of all that it has been assembled from hardware developed for the Nike X system originally intended for the defence of cities and rejected for this use by the present Administration and its predecessor. He said that Safeguard would not do the things expected of it. Even if it performed as it had been designed, however, the number of attacking missiles destroyed would be negligible compared with the cost of the system. He pointed out that the defence of each Minuteman complex would depend on a single radar set costing the best part of $200 million and that this part of the Safeguard system is so vulnerable to attack that a substantial part of the defensive missiles must be devoted to "defending the defense". He went on to argue that the computer system to which the management of an engagement would be entrusted is so complicated that even experienced computer programmers "have no faith that the system would actually work properly when called in, particularly since it would never have undergone full scale tests". For this reason, Dr Hornig concluded, Safeguard cannot be justified as a defence of Minuteman sites. Specifically, "the cost per silo defended would be many times the cost of each Minuteman saved" and would also be greater than the cost of the enemy missiles which could be intercepted with confidence, so that the chief consequence of Safeguard would be to persuade opposing nations to increase the number of attacking missiles.

But is Safeguard necessary as a protection against mainland China ? Since the United States will have overwhelming nuclear superiority over China for years to come, Dr Hornig said the question is "whether the Chinese would choose to commit suicide". For him, the threat of an outright attack by the Chinese on the United States is not to be taken seriously. In any case, he went on, Safeguard could not serve as a reliable protection even against the light Chinese attacks which might be theoretically possible in the late 1970s, partly because it has never been tested, partly because it is in any case a leaky defence, because a puny but prudent attacker would choose targets not yet protected by the early phases of Safeguard, and because even the Chinese would be able, in the late seventies, to confuse the Safeguard radars with extraneous objects.

Dr Hornig echoed the conclusions of his colleagues by saying first of all that he would, if left to himself, abandon Safeguard altogether and would certainly not continue it beyond the first phase of development, but that instead he would put some money into research and development. Similarly, he said, he would delay the deployment of MIRV and put his energy instead into the SALT talks.

Dr Wiesner had some tales to tell of how the United States Administration seems consistently to have overestimated the danger of the weapons in the hands of its potential opponents. He said that "we almost always react from fear—fear of the Russians and in the case of our leaders, fear of our own people", and explained that at the beginning of the Kennedy Administration in 1961, when it was learned that the supposed "missile gap" between the United States and the Soviet Union was more probably in favour of the United States, several people within the Administration (including himself) argued that the time had come to hold down the number of American missiles in the hope that the Soviet Union could be persuaded to do the same. In the end, however, he said, the Administration had agreed to build 950 Minuteman missiles and a force of 41 Polaris submarines. "The explanation given by Secretary McNamara for his recommendation was that because the Air Force recommended 3,000 Minuteman missiles, 950 was the smallest number Congress would settle for." He went on to say that much of the difficulty in arranging for a test ban followed, in his view, from that decision. The parallel with Safeguard and the SALT talks now under way was all too close.

Dr Wiesner also drew attention to the reports now widespread in the United States that construction of SS 9 missiles in the Soviet Union has been tailing off, which means that earlier estimates that there may be 500 of them by the mid-seventies are unlikely to be borne out. But even if there were 500 operational SS 9 missiles, Dr Wiesner argued, a surprise attack on the Minuteman force would leave roughly 270 American missiles intact, not to mention those in submarines and aircraft. "Actually Safeguard looks worse today than it did a year ago to some people—it always looked hopeless to me." It would provide little protection for the US deterrent and in any case such protection is unnecessary.

Inevitably, Senator Gore's interest was aroused by Dr Wiesner's tale of the Minuteman decision and by his statement that "I have seen more proposals for arms control destroyed by the compromises it is necessary to make within the government than by any other cause". He went on to say that in his days at the White House "we used to say that we had four fights on our hands for each new measure of arms control suggested—the Pentagon, Congress, the public and also, I suppose, the Russians". He supposed that Russian negotiators had similar problems, with the result that each delegation would turn up at a conference asking for something unreasonable in the hope of finding out what flexibility the other side would have.

The three Scientific Advisers said that their view of the ABM deployment now under way was shared by the rest of the scientific community. Dr Hornig said he had found a remarkable degree of unanimity and Dr Wiesner went so far as to say that there was very little disagreement with his point of view among the contractors actually building the Safeguard system.

TECHNOLOGY ASSESSMENT

Mr Daddario flies a Kite

A MAJOR threat to the security of the United States is posed by its increasing population, the rapid consumption of natural resources and the deterioration of the environment. Thus argue the advocates of a bill to establish for Congress an Office of Technology Assessment. Hearings on this proposal continued last week before the subcommittee on Science, Research and Development, chaired by Mr E. Q. Daddario, one of the sponsors of the bill.

The OTA would be governed by a board consisting of members of the legislature, seven members of the public appointed by the President, and the Comptroller General and the Director of the Legislative Reference Service of the Library of Congress. The OTA would be under the immediate control of its Director, also a member of the board. Under the bill, the OTA would assess the consequences of technology, present and future, either on request from the chairman of any Congressional committee, the board, or the Director. Its annual budget would be $5 million, which would have to include payments for contracts for assessments to be made by universities or industry when necessary. The advocates of the bill, by no means Luddites, seek a mechanism whereby expert judgment on complex technical issues can be presented to the legislature. The Administration gets advice from several sources but Congress is much less well supplied with information and help, in spite of the valiant work of the Library of Congress in recent years.

The subcommittee's inquiry so far has been thorough, and the witnesses agree that Congress needs the kind of service that Mr Daddario would father on it. But they disagree about the form which such an advisory body should take and even about the credibility of procedures of technological assessment as such.

Dr W. D. McElroy, Director of the National Science Foundation, last week endorsed the need but regretted that the putative Office of Technology Assessment would lack credibility—a great offence in Washington. He argued that technological assessment is so complex that it is virtually impossible to define what is meant by it, and that ignorance prevents a full assessment of the technological consequences of any decision. He said that the technological assessments which had been made so far have been concerned with only first-order effects—the immediate consequences of present decisions. Deeper judgments are harder to come by. If perfection is unattainable, however, imperfect practice is rife. Dr McElroy said last week that several institutions of government are already involved in technological assessment, and he suggested that the proposed office should be a supplement and not a substitute. In particular, he was clearly worried that the creation of the OTA might hinder activities which the National Science Foundation has under way already. He and other witnesses last week emphasized that there are comparatively few people who are able and willing to staff a body such as the OTA.

What are the alternatives? Dr McElroy suggested that the Reference Service of the Library of Congress should shoulder this responsibility, but Mr Daddario briskly retorted that the library has not so far carried out specific investigations and should not do so. Perhaps it would help the OTA, but that is all.

Putting more sand in the works, Dr McElroy also asked whether the OTA would be most useful as a link between the bodies already involved in technological assessment. He was anxious that multiple liaison between a research oriented OTA, institutions of the Administration and the NSF might be confusing. He suggested that in any case much of the required capability is allowed for under existing legislation, and cited as an example the National Environment Act of 1969, which created the Council for Environmental Quality. Dr McElroy also said that the NSF itself already has on hand several projects of technological assessment and is considering whether to establish a unit reporting to the director. The subcommittee may be expected to be duly sympathetic to the plea that the proposed OTA would have the undesirable result of establishing by statute the function of part of the internal organization of the NSF, but there is also the less generous interpretation that the NSF may seek to oppose the creation of another organization which may compete with it for public attention and influence.

Only lukewarm support for the bill came last week from Dr E. Wenk, of the University of Washington, who regarded the bill as inadequate. He suggested that the OTA should be independent not only of the Administration but also of the other branches of government, with a role not merely to advise but also to take decisions on technological matters, if necessary enforcing them in the courts. This clarion call was sharply refuted by the subcommittee. One member observed that, in effect, the head of this body would be an all-powerful technological Ombudsman. Mr Daddario remarked that, if effected, these proposals would be tantamount to the creation of a fourth branch of government, and in discussions with a later witness the subcommittee took the view that such drastic measures would neither be possible under the existing constitution nor, in any case, desirable.

Dr Wenk's vision of the OTA included a staff of some 300–400 with an annual budget in the region of $15 million. This was in sharp contrast with the previous estimate, from Dr McElroy, of 25–30 people, and Mr Daddario had to remind his witness that the bill provides for an annual budget of a mere $5 million, which must include payment for any investigations contracted out. The chairman also remarked that in any case he would doubt the ability of his subcommittee to steer through Congress a more demanding proposal. Although Dr McElroy's estimate is no doubt the more sober, this conflict does draw attention to one drawback of the bill—it is not yet clear just what problems would come under the aegis of the OTA and whether it would in fact have the capability to resolve them.

The subcommittee also dealt last week with the political framework within which the OTA would have to operate, prompted by the testimony of Dr E. Lyons, Chairman of the Department of History at Dartmouth College, and of Dr Dale Wolfle, the retiring Executive Officer of the American Association for the Advancement of Science. The intention of the bill is to strengthen the ability of Congress to exercise a critical faculty on technological issues, and the advocates of the bill argue that there is at present considerable scepticism among the public about the ability of governments to resist the demands of powerful pressure groups. The OTA could help in this respect, as it could also help to stimulate an informed public opinion.

GERONTOLOGY

Public Anxiety about Ageing

THE most recent sign of how exercised Congress has become by the problems of old people is the publication last week of a report of the US Senate Special Committee on Aging. The document is remarkable not merely for its thoroughness but for the way in which its detailed recommendations foreshadow legislation which is likely to transform the condition of old people in the United States. The starting point for the committee's analysis is a carefully documented demonstration of how ageing is increasingly to be equated with impoverishment, deepening with the passage of time. According to statistics compiled by the committee, the median income of families with the head older than 65 was 51 per cent of the median income of younger families in 1961 but only 46 per cent of it in 1967. Three out of ten people aged 65 and older are reckoned to have been living below the poverty line in 1966—in particular, one-quarter of older people living alone had an income of less than $1,000 a year in 1967. One obvious cause of difficulty is that retirement pensions from all sources tend now to amount to between 20 and 40 per cent of average earnings immediately before retirement. The committee calculates that in 1980 roughly half the then married couples will receive less than $3,000 in pension income and it points out that the tendency towards earlier retirement will decrease people's expectations of pension income. Moreover, the committee points out that economic growth, which provides working people with increasing amounts of money to spend, puts pressure on those who have retired while rising medical costs, only partly met by Medicare, will continue to increase.

Among the committee's radical recommendations for dealing with the problem of an ageing population is the suggestion that old people should share in the growth of the economy even though, after retirement, they no longer contribute directly towards it. The committee asks that there should be a social security system providing more liberal benefits than at present, including a widow's benefit equivalent to that to which retired men are entitled, better arrangements for insurance under the Medicare system, an extension of Medicare benefits to deal with prescription drugs for the treatment of chronic diseases, together with a number of reforms of the system (such as the relaxation of the requirement that a person must spend three days in hospital before extended care can begin).

These recommendations are very much in tune with the discussions now widely to be heard of the need for a national health insurance programme in the United States. The Senate Committee on Aging is also concerned with the nutrition of old people, known to be deficient in several well documented ways and not properly taken care of by devices such as the food stamp programme by means of which the indigent are provided with means of buying food cheaply. In much the same spirit, the committee argues the case for the use of urban transit systems in ways that will favour old people and more vigorous attention to the training of older people so as to equip them to stay at work until 65.

The committee complains that in the past year, the funds available for social programmes among old people have been reduced by the curtailment of the Federal budget. The committee is plainly hoping that many of its proposals will have reached the point at which they can be quickly implemented before or soon after the conference planned for 1971—and it is at the same time disconcerted to find that the work done so far in the planning of this conference has fallen short of what it considers necessary.

AIR TRANSPORT

Narrow Shave for the SST

THE project to build a supersonic civil aircraft in the United States narrowly survived defeat in the House of Representatives last week, by 176 to 162. The margin is narrow enough for those who have consistently opposed the project to hope that they will yet be able to bring it to a halt, although the chances are small that they will now be able to deny the Department of Transportation the $290 million in the budget for the coming financial year that has been earmarked for the development of the Boeing aircraft. Their next chance will come when the budget finds its way to the Senate a few weeks from now.

The recent history of the American supersonic transport will seem familiar to those who have followed the Concorde project in detail. Public money was first spent on the project nearly 10 years ago, when Congress authorized $11 million for study of the design. The original intention, fostered by President Kennedy, was that the Federal government should foot 75 per cent of the bill for development and that the total cost should not exceed $1,000 million. After competitive and parallel studies by Boeing and Lockheed, a consortium of Boeing and General Electric was chosen to build the commercial version of the aircraft in 1966. So far, $517 million of public money has been spent on the project, and the sum earmarked for the year ahead will carry the total over the ceiling suggested by President Kennedy in the early sixties.

Opposition to the project has been apparent in Congress for several years, and is now also apparent within the Administration. One of the most vigorous opponents has been Senator William Proxmire, whose economic subcommittee has been collecting evidence against the aircraft for the past five years. Since the beginning of the present Administration, there have been several inquiries into the desirability of continuing support for the project. An inquiry under the auspices of the Under Secretary for Transportation, Mr James Beggs, at the beginning of 1969, was inconclusive, and a review of the documents of that case is now known to have persuaded the President's Science Adviser, Dr Lee DuBridge, to say that he was not in favour of continued support for the project, chiefly on the grounds of public nuisance. A further study in Dr DuBridge's office seems to have confirmed this opinion, but the Administration as a whole decided to go ahead with building the supersonic transport in September last year.

As things are now, the Department of Transportation is the most consistent supporter of the supersonic transport within the administration, chiefly on the grounds that the aircraft will enable the United States aircraft industry to retain its technical leadership. One estimate is that the United States would lose a total of $16 billion by 1990 if other people's supersonic transports turn out to be successful but if the United States has not by then followed suit.

Annual Medallists

THE National Academy of Sciences at its 107th Annual Meeting this week has made the following awards: Dr KLAUS KEIL has been given the George P. Merrill Award for his work on meteorites—he is at present professor of geology and director of the Institute of Meteorites at the University of New Mexico. Dr EDWARD P. HENDERSON has been awarded the J. Lawrence Smith Medal for his lifetime's work for the division of meteorites at the Smithsonian Institution—he retired in 1966. DR EARL R. STADTMAN receives the academy's Award in Microbiology for his work on enzyme control mechanisms at the National Heart Institute. DR A. DALE KAISER has been awarded the US Steel Foundation Award in Molecular Biology for his work at Stanford University on the replication of virus DNA in infected cells. DR RAYMOND C. MOORE, of the University of Kansas, receives the Mary Clark Thompson Gold Medal for his work in editing the monumental *Treatise on Invertebrate Palaeontology*. DR THOMAS FRANCIS, professor of epidemiology at the University of Michigan until his death last October, was posthumously awarded the Jesse Stevenson Kovalenko Gold Medal for his involvement with the introduction of vaccines against virus diseases, particularly the Salk polio vaccine in 1954 and the influenza vaccine in the late sixties.

NATIONAL SCIENCE BOARD

Chairman for Science Board

THE National Science Board, the governing body of the National Science Foundation, last week elected as chairman for the next four years Dr H. E. Carter, Vice-Chancellor of the University of Illinois. Dr Carter will succeed Dr Phillip Handler, who became President of the National Academy of Sciences a year ago. Dr Handler remains a member of the National Science Board, but the election will remove the anomaly in which Dr Handler has been chairman of what is essentially a government agency, autonomous though it may like to think itself, and also president of the National Academy of Sciences and potentially a critic of government policy. Although this arrangement seems not to have prevented Dr Handler from speaking out on important issues, especially in the past few months on the reduction of the budget for university research, it has been plain even to his admirers that the arrangement could not continue indefinitely.

The appointment of Dr Carter will be watched for signs of whether he will be able to develop within the National Science Board the apparatus for appraising the development of public policy towards science and technology which has been thrust on the board in the past two years.

NEWS AND VIEWS

In Pursuit of the *Y* Chromosome

A FEVER of excitement among cytogeneticists is being whipped up by a chemical used to treat malaria. The chemical, quinacrine dihydrochloride, has a remarkable and as yet unexplained propensity for staining the stem end of the *Y* chromosome. It has already been used to spot the *Y* chromosome in human interphase cells and now the same has been done for spermatozoa. The technique will certainly afford a useful test of success to those who are trying to separate male and female determining sperm as a means of sex control. But the more immediate results will be in solving straightforward cytogenetic problems as well as in clinical medicine and genetic counselling. Moreover, it seems that not only does quinacrine mark others besides the *Y* chromosome but also that a whole range of different specificities may lie waiting to be discovered among other fluorochrome chemicals, particularly those based on the acridine ring. At the least the new technique will hasten the rate of progress in human cytogenetics and with any luck the next five years should see a number of fresh insights opened into the behaviour of chromosomes in cells.

The story began with the work of T. Caspersson and his colleagues who showed some two years ago that certain fluorescent derivatives of acridine stain the chromosomes of various organisms. Last year one of Caspersson's colleagues, Laura Zech, noticed that quinacrine mustard makes part of the human *Y* chromosome fluoresce particularly brightly. This illuminating finding has been taken up in several laboratories and important discoveries seem to be emerging rather faster than they can appear in print. Two months ago, for example, P. L. Pearson, M. Bobrow and C. G. Vosa demonstrated that the quinacrine staining test shows up the number of *Y* chromosomes in a human interphase (non-dividing) cell. This is an important time saver for those engaged in screening human populations for *XYY* males, the possessors of an extra *Y* chromosome who tend, as adults, to be found in prisons slightly more often than it seems they should be. Hitherto the only way of identifying the extra *Y* chromosome has been the laborious task of culturing leucocytes and doing a full karyotypic analysis. The quinacrine test is the exact counterpart of the simple stain for the Barr body which shows up the presence of extra *X* chromosomes and led indirectly to the discovery of *XYY* males in the first place.

The next two apples to fall from the tree are displayed in this issue (pages 959 and 961). Pearson and Bobrow have used the quinacrine technique to settle the admittedly somewhat academic issue, though one of thirty years' standing, as to whether it is the short arms or the stem of the *Y* chromosome that is associated with the *X* chromosome in meiosis; the answer is the short arm.

Peter Barlow and C. G. Vosa have used the stain on human spermatozoa with the result that they have distinguished, as many geneticists have dreamed of doing, the male determining spermatozoa from the female ones. Admittedly this will not lead tomorrow to methods of sex control, if only because the dye almost certainly kills as it distinguishes. But in any case the quinacrine stain, or others like it, should provide a simple test for assessing the various methods, whether based on centrifugation or electrophoresis, whereby people have sought to separate the two kinds of sperm. In the past the outcome of such methods has been uncertain because it could only be tested by breeding.

Animal breeders should hold their enthusiasm for a little while yet. Dr Barlow has said on the telephone that quinacrine now appears not to distinguish the *Y* chromosomes of bull spermatozoa since it stains all the chromosomes equally. It may be that man is unique among the mammals in this respect. But even if quinacrine is selective only in man, its use in this species is not restricted to the *Y* chromosome. Dr Pearson and his colleagues at Oxford have found that certain of the autosomes take up the stain in a way that depends on the individual. The acridine dyes certainly hold a lot of interesting secrets in store and in the coming months laboratories throughout the world will doubtless be fluorescing with excitement to find out just what these may be.

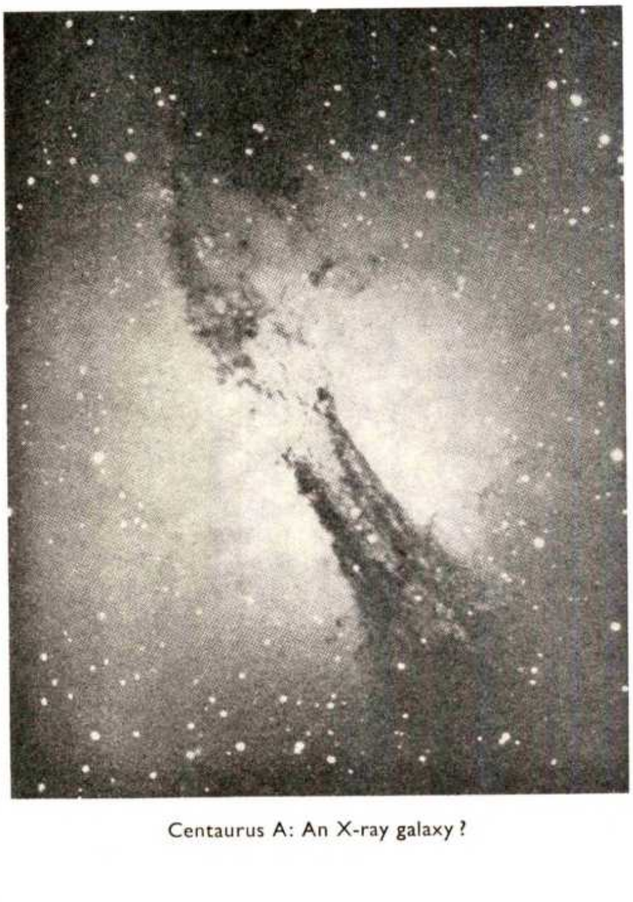
Another X-ray Galaxy ?

THE detection of X-rays from the radio galaxy Centaurus A by C. S. Bowyer's group at the University of California, Berkeley, brings to three the number of extragalactic objects with associated X-ray emission. There are, of course, thirty odd X-ray sources known to be probably within the Milky Way galaxy, of which the strongest appears to be Scorpius X-1. But Centaurus A at a distance of twelve million light years seems to be intrinsically of the order of 10^5 times more powerful than Sco X-1. With the result of Bowyer, M. L. Lampton and J. E. Mack indicating that the X-ray emission from the radio galaxy Centaurus A is approximately twice the radio emission, it seems that Centaurus A, one of the brightest objects in the radio sky, ought more properly to be called an X-ray galaxy.

Its peculiar appearance at optical wavelengths as much as anything has been the stimulus to observe Centaurus A with X-ray equipment, but so far the results have been inconclusive. The California telescope flown on an Aerobee rocket from Natal, Brazil, last summer measured a flux of 0·04 photons cm^{-2} s^{-1} making the X-ray emission from Centaurus A roughly a third of that from the X-ray galaxy M87. The third extragalactic X-ray object is the quasar 3C273, and all three were detected during the flight. Clearly the sensitivity of the California telescope coupled to the technique of concentrating on just three sources rather than allowing the telescope to scan across the sky tipped the balance in favour of the detection of Centaurus A.

The optical characteristic of Centaurus A is the dark lane running across the galaxy, but at radio wavelengths the pattern is the familiar one of a double radio source about three million light years across with the optical galaxy at the centre. In the case of Centaurus A, however, the optical galaxy marks the midpoint of a second double radio source which is much nearer the optical object. From the current dogma of exploding galaxies the inference is that Centaurus A must have exploded twice.

Another stimulus behind the search for extragalactic X-ray objects is to see whether sources outside the Milky Way can account for the isotropic X-ray background. This would require galaxies to emit something like 10^2 to 10^3 more energy in X-rays than at longer wavelengths. But with the ratio being more like fifty for M87 and about unity for Centaurus A this hypothesis now looks less probable than the alternative based on Compton interactions between cosmic ray electrons and the microwave background.



Centaurus A: An X-ray galaxy ?

SINGULARITIES

How to Spot a Black Hole

from our Cosmology Correspondent

INTEREST in the existence of "black holes" of collapsed matter, which was recently stimulated by Lynden-Bell's suggestion that such holes could be associated with quasars and galaxy centres, will receive a further boost as a result of the relative ease of some observational tests which have now been proposed by A. A. Wyller (*Astrophys. J.*, **160**, 443; 1970). Wyller is particularly concerned with the possible existence of black holes in globular clusters, where they may be responsible for the brightness excesses observed at the cluster centres. Postulating that a supermassive object has collapsed into a singularity in such a cluster, Wyller examines in detail spectroscopic and gravitational tests for such an object; these are marginally possible with present-day techniques, so that there is every chance of a positive result being achieved as future developments make observations easier.

The holes considered have masses comparable to a few per cent of average globular cluster masses, which are themselves $\sim 10^5$ $M_\odot$, so that their radii are in the range 0·01–1·0 $R_\odot$. For the particular case of 47 Tuc, where the spectroscopic possibilities are best, as a result of the cluster's large size and proximity to the solar system, an analysing diaphragm of 2″ would give a sampling radius of 10^6 $R_\odot$ about the hypothetical black hole, in which range it should be possible to detect the contribution of orbital Doppler shifts in the spectra of the stars close to the central singularity in the line spectra. Measurement of a gravitational redshift would be more difficult, but this might prove detectable as a steady component while the Doppler component varies cyclically over a few years. Wyllers' calculations suggest that the steep density gradient inside a globular cluster ensures that, for 47 Tuc at least, contributions to the total line profile in the line of sight from stars perturbed by the black hole and those unaffected by it should be roughly comparable.

The technological advances utilized by Weber in his search for gravitational radiation make this a plausible alternative means to observe black holes. With two revolving black holes at the centre of a globular cluster, Wyller shows that the rate of gravitational radiation can be much higher than that expected from other phenomena, both in the Earth mode frequency (1 cycle per hour) and higher frequencies up to ~1 cycle per second. Again treating 47 Tuc in detail, he predicts a flux at the Earth of $3\cdot7\times10^5$ erg cm^{-2} s^{-1}, compared with 3×10^{-5} erg cm^{-2} s^{-1} for the flux from a white dwarf pair rotating every 100 s at a distance of 100 pc from the Earth. Even more pronounced effects might arise from the galaxy centre type of black hole discussed by Lynden-Bell and others.

Ap STARS

Anomalous through Diffusion

from our Observatories Correspondent

THE suggestion that the composition anomalies in the spectra of Ap stars have resulted from diffusion in the surface layers will be greeted with interest by students of these peculiar stars (G. Michaud, *Astrophys. J.*, **160**, 641; 1970). Ap stars are characterized by abnormally strong lines of certain elements, particularly silicon, manganese, chromium, strontium and the rare earths, while other elements—notably helium, oxygen and carbon—have abnormally weak lines. Known for sixty years, Ap stars constitute about twenty per cent of all main sequence stars with effective surface temperatures between about 8,000 K and 20,000 K, and are only found in this range. There is, however, no widely accepted theoretical understanding of their properties.

In the early 1950s, H. W. Babcock found that Ap stars had large surface magnetic fields, typically between 1 and 10 kgauss, and as high as 34 kgauss in one case. He found that the fields often varied, on a time scale ranging from days to years. Later work by Babcock and G. W. Preston produced evidence that the variations are periodic, that the period is the rotation period of the star and that the axis of the magnetic field (usually not a simple dipole) is inclined to the rotation axis. Other variations in the spectra of Ap stars have led to the idea of localized patches of abnormal composition on their surfaces.

The anomalies in composition are very pronounced and during the past fifteen years spectroscopic analyses have demonstrated correlations between the behaviour of certain elements. For example, in some Ap stars the rare earth elements are overabundant by factors of about 10,000, while oxygen is deficient by a factor of about 100. There is also evidence that the character of the abnormal compositions is related to the surface temperature. For example, stars characterized by an abnormally high abundance of Mn are only found with surface temperatures of about 12,000 K.

Suggesting that the composition anomalies in the spectra of Ap stars have resulted from diffusion in the surface layers, Michaud adopts the view that the large magnetic fields, the origin of which he does not consider, stably stratify the outer layer against mass motions such as convection. (An effect of this kind can be seen in sunspots where the magnetic fields damp down the solar granulation pattern.)

Hydrogen provides the bulk of the material in a stellar atmosphere. In a stably stratified atmosphere heavier elements diffuse downward under the gravitational field of the star. Michaud shows that the time scale for this gravitational settling is only 10^4 years, much less than the 10^8 years which the star spends in the main sequence. The tendency to sink is opposed by radiation pressure, however. This may act at the discrete frequencies of spectral absorption lines or in the photoionization continuum of any particular element. Whether or not the levitation due to radiation pressure wins over the gravitational settling depends on the temperature of the star and the details of the atomic spectrum of a particular ion. Thus an element may sink at one temperature when it is neutral and be pushed up at another temperature at which it is ionized.

Michaud has calculated the expected behaviour of several elements as a function of temperature, and found in many cases a satisfactory agreement with observation. In particular, he explains why stars with an excess of Mn are found with surface temperatures of about 12,000 K. But Michaud's diffusion mechanism may not be the full story. Spectroscopists have found lines of very heavy elements—mercury, platinum, osmium and perhaps even uranium—in some Ap stars, and there is evidence that the isotopic compositions of some of these elements are abnormal, in the sense that the neutron-rich isotopes are overabundant compared with terrestrial samples. This could not be attributed to atomic diffusion and would point to a nuclear origin for the composition anomalies of Ap stars. Specific nuclear theories, proposed by Hoyle, Fowler and the Burbidges, had some unsatisfactory features but no doubt the whole situation will be reassessed in the light of these new developments.

OCEANS

New Crustal Layer

from our Geomagnetism Correspondent

IT is generally accepted that the lowest layer of the Earth's oceanic crust, immediately above the Mohorovičić discontinuity, has a seismic velocity of about 6·8 km s^{-1}. This is the crustal layer with the highest velocity, and contrasts with the uppermost layer of the mantle which has a velocity greater than 8·0 km s^{-1}. Velocities between these figures are not unknown; a layer of seismic velocity 7·0–7·5 km s^{-1} is a feature of mid-oceanic ridges, some ocean margin regions and certain trenches, but velocities in this range have not been regarded as typical of ocean basin structure.

It now looks as if this view must be amended. Maynard (*Science*, **168**, 120; 1970) has produced unequivocal evidence that a basal crustal layer with velocities in the range 6·9–7·6 km s^{-1} (average, 7·3 km s^{-1}) exists below the Pacific Ocean in the region between the Marshall and Hawaiian Islands. Refraction seismology leaves no doubt that the new layer exists in this region—and its extent (1,000 km) and thickness (several kilometres) suggest that it is probably widespread throughout the South and Central Pacific.

But why has it been missed for so long ? One reason apparently is that the new intermediate layer is normally masked as a second arrival. Maynard overcame this problem by using an air cannon as a repetitive signal source in conjunction with sonobuoys and precision echo sounder recorders. The repetitive signal resulted in an extremely high density of data; and the recorders were arranged for phase correlations. This revealed refraction arrivals which are usually not recognized, either because they never appear as first arrivals at all or because they appear only in the form of short segments of first arrivals at a distance where data from explosive sources are widely spaced.

Further evidence for a wide geographical coverage of the new layer comes from refraction experiments carried out since Maynard's pioneering work. D. M. Hussong and his colleagues, for example, are known to have found the same layer in the deep ocean north of Hawaii and 180 km west of San Francisco, although full details have not been published. If the layer is widespread, however, the implications are interesting. For one thing, it would mean that there is a widespread change in composition or a polymorphic transition at the interface between the two lowest crustal layers. This would imply that the mean density of the Earth's crust must be higher than previously supposed—an adjustment which would affect depths determined from gravity anomalies and possibly even density distributions consistent with the Earth's moment of inertia.

METEOROLOGY

Droughts and Fires

from our Geomagnetism Correspondent

EVERY schoolboy knows that droughts are liable to result in vegetation fires. What is less obvious is that the fires themselves contribute to the drought, especially in tropical and sub-tropical climates. The reason for this is that the tropical cumulus clouds, in common with all clouds, require nuclei to condense on before rain can fall. When such condensation occurs raindrops begin to form on nuclei ranging from 0·1 to 1 μm in diameter but must grow to about 400 μm before they fall to Earth. If a large excess of nuclei is present, each nucleus will compete for the available water, and there is a real possibility that none will reach the critical size.

Burning vegetation produces many small particles, all potential cloud nuclei. Just how many particles are formed has been the subject of an investigation of the 1967 drought in Florida by Ronald J. Holle of the Experimental Meteorology Laboratory (ESSA) in Miami. During the drought from April 1 to May 15, at least 52,000 acres of vegetation burned within 115 miles of Miami. The frequency of fires was substantially higher than usual, but rainfall was observed only twice in the 46 days of drought—an extremely low total of only 0·15 to 0·30 inch which fell only on non-fire days.

What, then, did the fires contribute to this situation ? There are five major types of natural vegetation in Florida. Some 3,500 square miles are covered by everglades marshes, much of which is only saw grass although some areas have herbs, bushes, tree islands and sloughs as well. The surface is normally flooded with algae covering as much as 50 per cent of the water to a depth of about an inch. When a drought comes the water table lowers and the dry, combustible algae cling to the saw grass. Another 3,300 square miles are pine forest—open woodland lying partly on rock and intermingled with herbs, saw palmettos, shrubs, other hardwoods, tropical hummocks, cypress swamps, prairies and bay tree swamps. Grassland covers 850 square miles, open scrub cypress a further 660 square miles, and wet to dry prairie marshes make up 1,450 square miles.

Holle collected samples of each of these types of vegetation, burned them in controlled laboratory conditions and measured the cloud condensation nuclei produced. The materials burned were in a closed chamber in conditions simulating both open burning with abundant oxygen and confined burning resulting in slow combustion or smouldering. Each gram of vegetation burned produced between one billion (10^9) and ten billion condensation nuclei ! Slow burning produced about twice as many nuclei as fast burning, and algae, a major fuel in fires during periods of drought, produced twice as many nuclei as any other component. Holle is thus justified in his conclusion that fires can reduce rainfall from large cumuli and substantially reduce the rain falling from smaller clouds in the immediate vicinity of the fires.

It is therefore instructive to speculate on the effect of man-made fires. Jamaica, for example, suffers from droughts which may well be aggravated by the burning of cane fields. Cane fields are also burned in south Florida between November and April. In 1968, 168,000 acres of cane were burned in Palm Beach County alone. Whether fires on such a massive scale can actually produce a drought is still an open question; but certainly fires which occur during a drought can prolong the dry period.

PARTICLES

Cutting Tachyons down to Size

ONE of the loose ends of special relativity that has been achieving some attention in recent years is the idea that particles that travel faster than light cannot be ruled out by the special theory of relativity. Since the suggestion was first sported in 1962 there have been several attempts to contrive evidence to support it. A new clue as to why such faster-than-light particles, called tachyons, may be hard to find if they are not indeed a mathematical figment has come from an unexpected corner of physics, namely a model of a dislocation.

S. I. Ben-Abraham (*Phys. Rev. Lett.*, **24**, 1245; 1970) has drawn attention to the analogy between a model of dislocations developed by Frenkel and Kontorova and the theory of relativity. In this model the atoms move under harmonic forces between neighbours and a sinusoidal interaction with a rigid substrate, giving solutions which have been found to include supersonic excitations called positive and negative antidislocations. He sees the propagation speed along a chain of atoms—the velocity of sound—playing the role of the velocity of light and the supersonic solutions as being analogous to the tachyons attributed to relativity theory. A so-called tachydislocation is then described by a chain of atoms most of which sit on potential crests.

Ben-Abraham points out that at moderately supersonic speeds such excitations are highly unstable and have not been observed. The concentration of energy in such an excitation would give the appearance of a massive object in the solid, and in one dimension a boundary would exist dividing regions of subsonic and supersonic space. Ben-Abraham suggests therefore that as tachyons could only exist in regions inaccessible to normal subsonic particles this provides an indication that they are unlikely to occur in practice.

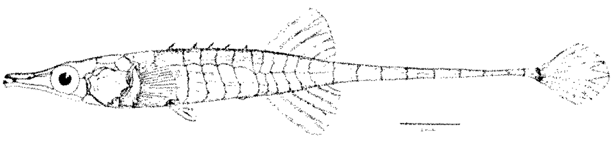
SYSTEMATICS

Paradox may be Solved

from our Marine Vertebrates Correspondent

SYSTEMATIC ichthyologists have made fine play with the relationships of the seahorses, pipefishes and their allies, and the sticklebacks. These creatures have been placed at almost opposite ends of the teleost classification, brought together, and then united in one order. One of the gems that confirmed the unitarians in their belief in a single order was the discovery in Lake Indawgyi, Burma, in 1926, of an enigmatic small fish later to be named *Indostomus paradoxus*. This seemed to bridge the two groups, as a stickleback-like pipefish with the long snout and body armour of the pipefishes, and a series of isolated spines along its back as in the sticklebacks. Now a study by K. E. Banister (*Bull. Brit. Mus.* (*Nat. Hist.*) *Zool.*, **19**, No. 5, 179; 1970) suggests that these resemblances are purely superficial and *Indostomus* is not related to either group.

Originally *Indostomus* was placed in the pipefish order Solenichthyes by its discoverers Prashad and Mukerji (*Rec. Indian Mus.*, **31**, 161; 1929), and Bolin later confirmed this (*J. Wash. Acad. Sci.*, **26**, No. 10, 420; 1936), although differing in his assessment of relationship within the family. Berg's classification of fishes lumped *Indostomus* in with his pipefish order Syngnathiformes, but more recently Greenwood *et al.* (*Bull Amer. Mus. Nat. Hist.*, **131**, No. 4, 339; 1966) placed it in a holdall order of Gasterosteiformes. The basic difficulty with *Indostomus* was two-fold: first, its anatomy had never been properly studied or described, and second, because it was a highly modified fish its systematic position was unclear.



Indostomus paradoxus.

The first of these difficulties has now been overcome with Banister's detailed examination of the skeleton. He finds fifteen major features in which *Indostomus* differs from the sticklebacks and their most primitive family the Aulorhynchidae, while the external body armour and pronounced snout are only superficial resemblances to the pipefishes. Banister's conclusion on the second point is that this curious little fish is sufficiently distinct to be placed in a new order Indostiformes, of which it is the sole member. The position of this order within the fish system, however, seems to be if not a paradox then as enigmatic as ever, and a tentative situation near the clingfish order Gobiesociformes is proposed.

But what of the fish itself? Banister's report concludes with a fascinating account of this peaceful little fish by Alan Tubb of the UN Food and Agriculture Organization in Thailand, who collected 140 specimens and kept them alive in aquaria. From these notes (the first on the behaviour or biology of the species) *Indostomus* can be seen as quiet, peaceful and generally slow-moving, although it can be swift when alarmed. It feeds entirely on living animal food, slowly stalking its prey with fins depressed, except for the fluttering of its pectoral fins. In spite of its seemingly rigid body armour *Indostomus* is remarkably flexible and seems to be able to move its head at an angle of some 10° to the body in all directions, an unusual accomplishment in a fish. Evidently *Indostomus* is a fish well endowed with surprises for the ichthyologist.

EMBRYOLOGY

Very Early Mammals

from a Correspondent

THE successful manipulation of the mouse blastocyst provided the topic for one of the most impressive contributions to the Schering symposium on intrinsic and extrinsic factors in mammalian development, held in Venice from April 20 to 24. Dr R. L. Gardner (University of Cambridge) reported that microsurgical techniques can be used to separate the trophoblast from the inner cell mass (ICM), and that the two components are markedly different in their behaviour and growth potential after transfer to the uteri of pseudo-pregnant recipients. Thus, grafts of trophoblast alone can induce decidualization, but otherwise fail to proliferate. By contrast, fragments of ICM develop within the host and produce normal young; pairs of such ICMs can be fused *in vitro*, even when their ages differ by twenty-four hours. Blastocysts and mere trophoblastic envelopes can also serve as carriers of an ICM of a different genotype, with subsequent mixing of their cells and formation of viable chimaeras. Such blastocysts "reconstituted" after *in vitro* manipulation provide a powerful new system for examining the diverse interrelations of trophoblast, foetus and the maternal environment. Gardner's results also suggest that the number of cells from which the embryo proper develops may be remarkably small.

The extraordinary resilience and ability to fuse of cleaving mouse eggs were demonstrated on film by Professor J. G. Mulnard (Brussels), while Dr W. K. Whitten (Bar Harbor) reported his highly successful technique for culturing pre-implantation embryos in an exactly defined chemical medium, with a high yield of normal mice following transfer to the uteri of pseudo-pregnant hosts.

Dr T. G. Baker (University of Edinburgh) provided convincing ultrastructural proof that the mammalian oocyte in the prolonged diplotene stage of meiotic prophase is not, as too often assumed, resting, but synthesizes ribonucleoprotein and actively contributes to the growth of the surrounding follicle.

The importance of the biochemical milieu encountered by the pre-implantation blastocyst was stressed by Drs H. M. Beier (Marburg, Germany) and J. C. Daniel (Colorado). They have shown that during this phase the intrauterine fluid in the rabbit has a high protein content, partly derived from the maternal plasma and partly from the endometrium itself. Among the specific fractions is a glycoprotein ("uteroglobin" or "blastokinin") which is present in intrauterine and blastocoelic fluid and may promote embryonic growth. No comparable fraction has so far been isolated from human uterine secretions.

The complex system of cellular interactions between the first stage of "induction" and the fully differentiated tissue may soon come into closer focus. This was the implication in the contribution from Professor H. Tiedemann (Berlin) who described the isolation of a protein fraction from chicken embryo which can induce the formation of endoderm and mesoderm in amphibians, but which is counterbalanced by an inhibitor, an acidic protein. The study suggests that transcription of RNA is involved in differentiation and that hormones may take part in the later morphogenetic stages.

PROTEINS

Primordial Muscle

from our Molecular Biology Correspondent

THE presence of contractile protein systems in such nondescript objects as amoebae and slime moulds, not to say in fibroblasts and leucocytes, is now well established. In several cases actin-like and myosin-like proteins have been dredged from the cytoplasm, and it has been conjectured that something very akin to muscular contraction may underlie the process of cytoplasmic streaming, which is the basis of the lethargic locomotion of which these organisms are capable.

The properties of the myosin from a slime mould, previously isolated by Hatano and his colleagues, have been studied recently by Hatano and Ohnuma (*Biochim. Biophys. Acta*, **205**, 110; 1970). In agreement with Adelman and Taylor (*Biochemistry*, **8**, 4976; 1969), they find that the protein has a sedimentation coefficient little different from that of rabbit muscle myosin. The viscosity, however, though high, is considerably less than that of muscle myosin, and it is inferred that the presumed rod-like part of the molecule is either shorter or more flexible. The most distinctive difference between the two proteins, as Adelman and Taylor also noted, is that when the salt concentration is reduced no filaments are formed; instead some low aggregates, up to perhaps the tetramer, can be discerned in the ultracentrifuge. The amino-acid compositions are very similar, with the notable exception that cysteine, of which there are enzymatically vital residues in rabbit myosin, is absent in the slime mould protein. The ATPase activities, according to Hatano and Ohnuma, are comparable, but Adelman and Taylor find three times higher activity in the slime mould enzyme. There is also disagreement about the effect of magnesium, which Hatano and Ohnuma say inhibits the ATPase, but which Adelman and Taylor say does not.

The broad parallel between the actomyosin of vertebrate muscle and the putative contractile system of the slime mould has been placed on a much surer footing by two new studies with the electron microscope. Nachmias, Huxley and Kessler (*J. Mol. Biol.*, **50**, 83; 1970) have shown that the actomyosin of this creature occurs as long filaments, some 50 Å thick, looking very like actin, and bearing at intervals formations of the characteristic arrowhead shape seen in mixtures of authentic F-actin and myosin. There are also thin fibres, joining the filaments at irregular intervals. When ATP was added all the attached bodies were released, leaving isolated filaments with the beaded structure of actin. Preparations of the actin-like protein had a thickness of 40–50 Å, which is less than that in muscle actin, and a 360 Å periodicity. The most striking feature of this work is the appearance of indubitable, though somewhat irregular, arrowhead structures on addition of rabbit myosin subfragment-1 (the isolated globular heads). That the slime mould actin reacts with mammalian myosin was inferred some time ago, but the present results show that the mode of interaction is the same as in muscle, and that the binding sites of the actin—both for myosin and for its own neighbours—have been conserved throughout evolution.

A parallel study, this time on an amoeba, accompanies this paper (Pollard *et al.*, *ibid.*, 91). The actin again appears as beaded filaments, some 60 Å in diameter, and in some of the pictures the twisted double helical structure can be made out, with a period of 370 Å. When the actin was mixed with rabbit heavy meromyosin (myosin with a proteolytically truncated shaft), good regular arrowheads were seen at intervals of 370 Å along the filament. A similar appearance is presented by the filaments of the intact cell on addition of heavy meromyosin, these measuring moreover about 80 Å across, just like the thin filaments of rabbit muscle. It thus seems that in spite of some obvious differences in the myosins, the mechanochemistry of these primitive cells is indeed closely related to that of muscle, and that different systems of motion evolved from a common precursor.

NITROGEN FIXATION

Proteins and Associations

from a Correspondent

ANOTHER step forward in the understanding of biological nitrogen fixation has been the recent isolation of the second protein associated with nitrogenase, the enzyme complex involved in this process. Nitrogenase consists of two protein components, no matter from what organism it is isolated. L. E. Mortenson's team at Purdue University obtained the first almost homogenous preparation of one of these—the oxygen-sensitive, non-haem iron protein from *Clostridium pasteurianum*—90 to 95 per cent pure. It had a molecular weight of 40,000 and contained two iron atoms and two labile sulphur atoms per molecule (*Biochim. Biophys. Acta*, **172**, 106; 1969). The second protein associated with nitrogenase is not particularly oxygen-sensitive; it has been known for some years to contain molybdenum as well as iron and labile sulphur, but it has been difficult to purify because of its disposition to aggregate and disaggregate. A significant advance has been made, according to preliminary reports, by R. W. F. Hardy's group at Dupont de Nemours, Wilmington, Delaware (*Biochim. Biophys. Res. Commun.*, **39**, 90; 1970).

They have obtained crystals of the Mo–Fe protein from *Azotobacter vinelandii* as needles which appear when the ionic strength of the solution is lowered. This is an unusual procedure for crystallizing proteins: high ionic strengths, or at least lowered water activities, tend to be favoured among practitioners of the art (which tends to have culinary as much as scientific overtones). The Dupont group's product has one or two peculiar properties: although the solution is intensely brown, the crystals are white. The molecular weight exceeds all earlier claims, being nearly 300,000, and the crystals contain approximately two Mo atoms, forty Fe atoms, forty cysteine residues and thirty labile sulphur atoms. Though crystallinity is not, among proteins, an absolute criterion of molecular homogeneity, the prospect of authentic crystals of one of the components of nitrogenase opens exciting vistas for structural studies.

On the more biological front, the use of the acetylene reduction technique continues to revolutionize ideas about the distribution of nitrogen-fixing organisms. Several groups of microorganisms which were believed to be able to fix nitrogen have now been struck off the list; hitherto unsuspected ones are being discovered in strange environments. Perhaps the strangest case was described by F. J. Bergersen and E. H. Hipsley of the CSIRO Division of Plant Industry, Canberra (*J. Gen. Microbiol.*, **60**, 61; 1970), who found nitrogen-fixing bacteria of the *Klebsiella* group in the intestines of pigs, guinea-pigs and humans. Among the humans were not only Europeans from Canberra, but also natives from the Morobe district in the Territory of Papua–New Guinea; natives who live on a very low nitrogen diet, based principally on sweet potatoes. These observations raise once more the idea, never completely dismissed, that nitrogen-fixing bacteria can contribute to the nutritional status of animals, including man, when the dietary nitrogen is low. Bergersen and Hipsley showed that a guinea-pig which had been fed a diet low in nitrogen for several days reduced acetylene, slowly but definitely, when exposed to this gas in an enclosed chamber. This experiment shows that the nitrogen-fixing bacteria in its intestines were actually active, though their relevance to its nutrition is still an open question. All the well established nitrogen-fixing associations have been between bacteria and plants, and the leguminous association, which is perhaps the best known, has been peculiar because neither the plant nor the microorganism can be persuaded to fix nitrogen separately. At the April meeting of the American Society for Microbiology, Hardy's group reported use of the acetylene reduction technique to demonstrate fixation *in vitro* by microorganisms growing with isolated plant cells. Rapidly dividing cultures of root cells from soya bean were inoculated with *Rhizobium japonicum*; an association was formed which could be observed by electron microscopy and which reduced acetylene at about 1 per cent of the rate found with intact soya bean root nodules. As Hardy and his colleagues recognize, these experiments open, for the first time, the prospect of studying the effects of plant hormones on this curiously obligatory symbiosis.

PERCEPTION

Detectors in Human Visual System

from our Neurophysiology Correspondent

EVIDENCE has recently been published for the existence of orientation and size detectors in the human visual system (Campbell and Maffei, *J. Physiol.*, **207**, 635; 1970). This gives firm support to the possibility of linking neurophysiological and psychophysical data.

Campbell and Maffei showed their subjects gratings on an oscilloscope face. These were fields with brightness sinusoidally modulated spatially, giving the appearance of fuzzy, alternating light and dark bars. Previous experiments had established psychophysical thresholds for seeing a grating at various contrast levels and spatial frequencies (discussed in *Nature*, **224**, 311; 1969). The psychophysical threshold was defined as that contrast level at which the subject could correctly report the orientation of the grating, which implied that he could clearly distinguish it from a uniformly bright field of the same mean luminance. To obtain a neurophysiological measure Campbell and Maffei presented the gratings as before, but reversed their contrast eight times a second. They recorded the evoked potential to this stimulus between two scalp electrodes above the visual cortex. The evoked potential was filtered so that its power at eight cycles, the stimulus frequency, could be measured. Each experimental datum was therefore an evoked potential amplitude for a grating of particular spatial frequency at a given contrast level. To obtain a measurable evoked potential amplitude it was necessary to average the response to several hundred presentations, improving the signal-to-noise ratio. This limited the experiment to stimuli well above threshold contrast as the nearer the threshold the longer the averaging time needed for a measurable amplitude.

Their results show that amplitude is linearly related to log contrast, as would be expected if Weber's law applied. (Weber's law states that the least discriminable stimulus increment is a linear function of background stimulus intensity.) Further, linear extrapolation to zero amplitude gives a value of contrast agreeing well with the psychophysically measured contrast thresholds at all spatial frequencies used.

This technique was also used to investigate the effect of adapting the retina to one grating on the visibility of a second. There was no decrement in visibility if a grating of the same spatial frequency as the adapting grating was presented in an orientation such that its axis was at least 15° from the axis of the adapting grating. With orientation differences between 0° and 15° there was an approximately linear increase in the amplitude of the evoked potential of the second (test) grating. Similarly, gratings of the same orientation but at least one octave apart in spatial frequency showed no decrement in visibility. Reducing the frequency produced an increasing interaction leading to a decrease in the visibility of the test grating.

Campbell and Maffei have thus given clear evidence of many channels sensitive to frequency and orientation, which are largely independent. Subsidiary results show that the foveal and extra-foveal distributions of these are different. Where relevant psychophysical data are available they agree with data from these experiments.

Three Hundred Years of Botany in Edinburgh

by
J. A. RATTER
Royal Botanic Garden,
Edinburgh, 3

This week the tercentenary of the Royal Botanic Garden, Edinburgh, is being celebrated with, among other things, the opening of an exhibition hall given by an anonymous donor. This article traces the fortunes of the Garden during the past three hundred years.

LIKE many other European botanic gardens, the Royal Botanic Garden, Edinburgh, can trace its origin to a physic garden founded for the service of medicine. The original garden consisted of an unpretentious plot at Holyrood Abbey, measuring only 40 foot square, which was rented for growing medicinal plants by two friends, Dr Robert Sibbald (Fig. 1), the first Professor of Medicine in the University of Edinburgh, and Dr Andrew Balfour. Continuous development of this garden, involving several changes of site and status, has resulted in the Royal Botanic Garden of today.

Maintenance of the original garden was entrusted to a youth called James Sutherland who was to have a vital influence on its development and future history; by his industry the garden developed and soon required the addition of more land which was obtained at separate sites in 1675 and 1695. Sutherland became one of the great botanists of his day and was appointed Professor of Botany in the University of Edinburgh in 1695; thus began the union in one man of the duties of Keeper of the Botanic Garden and Professor of Botany which, apart from the years 1706–1739, was maintained until 1956. In 1699 Sutherland was further honoured by becoming King's Botanist under a warrant of King William III, and in 1710 Queen Anne created for him the post of Regius Professor of Botany.

SIR ROBERT SIBBALD BART M.D.

Fig. 1. Sir Robert Sibbald, first Professor of Medicine in the University of Edinburgh, one of the two co-founders of the Royal Botanic Garden, Edinburgh.

Following Sutherland, the Garden had two outstanding Regius Keepers during the eighteenth century: Professors Charles Alston (1716–1760) and John Hope (1761–1786). A manuscript copy of Hope's student lectures, in the library of the Garden, reveals that he was an experimental plant physiologist of great ingenuity and well informed on the research of botanists throughout Europe. Apart from plant physiology his other great interest was systematic botany, and unlike some of his contemporaries he was a strong advocate of the system of Linnaeus. He encouraged his students to explore and investigate the flora of Scotland and annually gave a gold medal for the best student's herbarium. Undoubtedly Hope and his students were the pioneer investigators of the Scottish flora, and he himself greatly influenced the work of Lightfoot who published his *Flora Scotica* in 1778. From the point of view of the future history of the Botanic Garden, Hope's greatest achievements lay in securing a permanent endowment for its maintenance from the Crown and in obtaining a new site in Leith Walk to which the plant collections were transferred in 1763.

The years from 1786 to 1820 were rather unpropitious for the history of the Botanic Garden, although some members of the staff were active in the study of the Scottish flora, most notably the great Scottish taxonomist, horticulturist and plant explorer George Don who was Principal Gardener from 1802–1806. By 1820 the Botanic Garden at Leith Walk was in a parlous state: its five acres were too small to accommodate the collections accumulated by the enthusiastic and able Principal Gardener, William McNab, the glasshouses were in dilapidated condition and the expansion of the city had encroached closely around it. Because of this a fourteen and a half acre site at Inverleith was purchased. This site formed the nucleus of the Botanic Garden of today, and adjacent properties were later acquired and added to it so that a garden of 68 acres was eventually produced. Two years were taken to complete the transfer of the plant collections from the Leith Walk Garden to Inverleith. Well established trees, some more than forty feet tall, were moved by means of a transplanting machine (Fig. 2), akin to those of today, which was designed by the resourceful McNab. He published an account of the successful tree transplanting operations in 1830 in an important paper, "Hints on the Planting and General Treatment of Hardy Evergreens in the Climate of Scotland".

Botanical Society of Edinburgh

The Regius Keeper from 1820 to 1844 was Professor Robert Graham who was forced to devote much of his efforts to making representations to improve the totally inadequate Government grants made to the Garden. These representations bore fruit, and in 1834 he was able to

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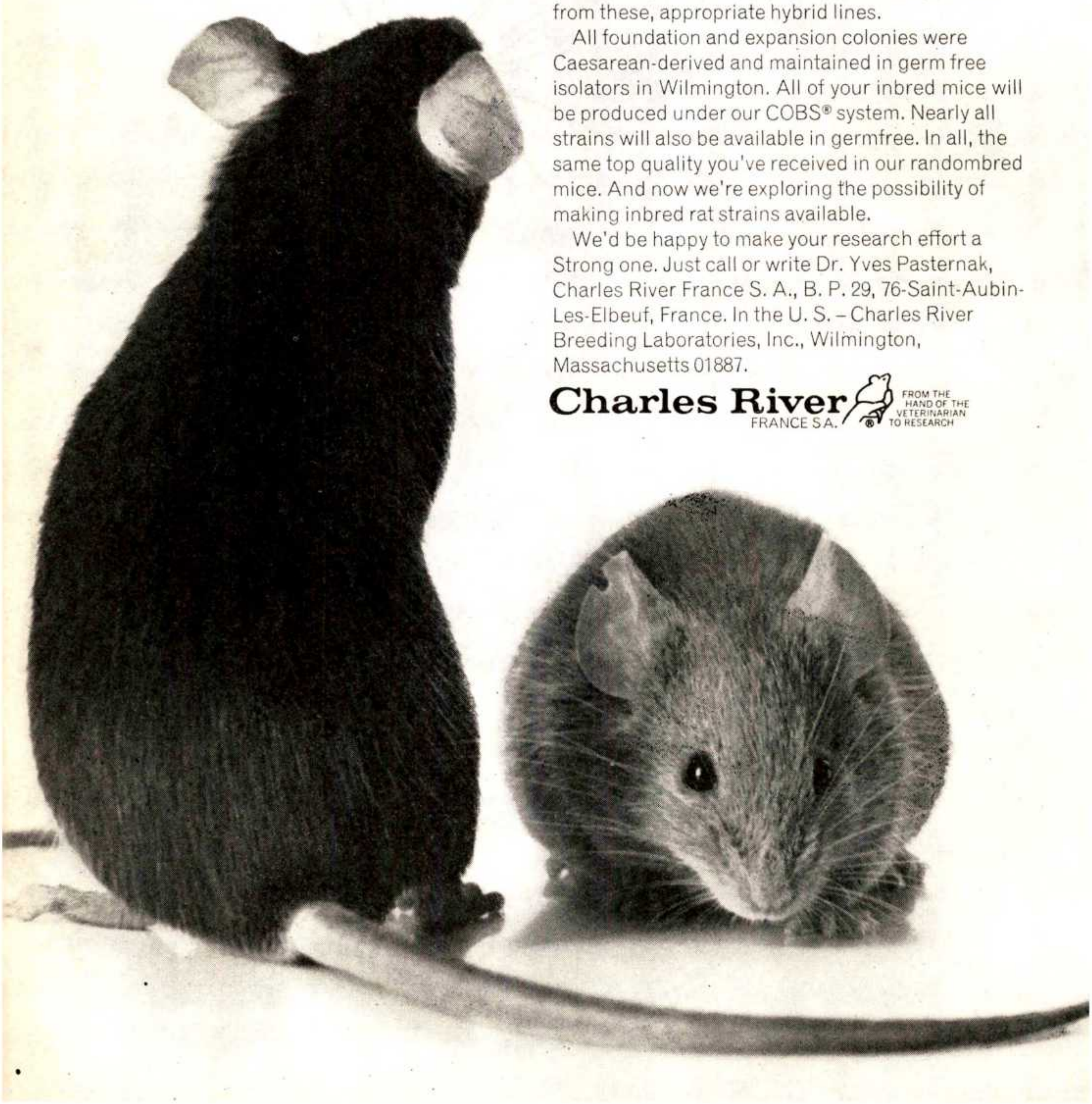
FROM THE HAND OF THE VETERINARIAN TO RESEARCH



open a new tropical palm house, the largest of its type in Britain, the construction of which had been financed from Government funds. Perhaps Graham's greatest achievement was the part he played in the founding and early development of the Botanical Society of Edinburgh. The Society was founded in 1836 and Graham was its first President, and strove for its success to the end of his life. Throughout its auspicious history the Botanical Society of Edinburgh has had a symbiotic relationship with the Royal Botanic Garden: the donation in 1863 of its herbarium of many thousands of specimens and in 1872 of its valuable library formed the nucleus around which the extensive herbarium and library of the Royal Botanic Garden have been built. Robert Graham would surely be happy if he could see the embryonic society which he nurtured as it is today—a body with a large, active, international membership sponsoring an important part of the tercentenary celebrations of his Botanic Garden.

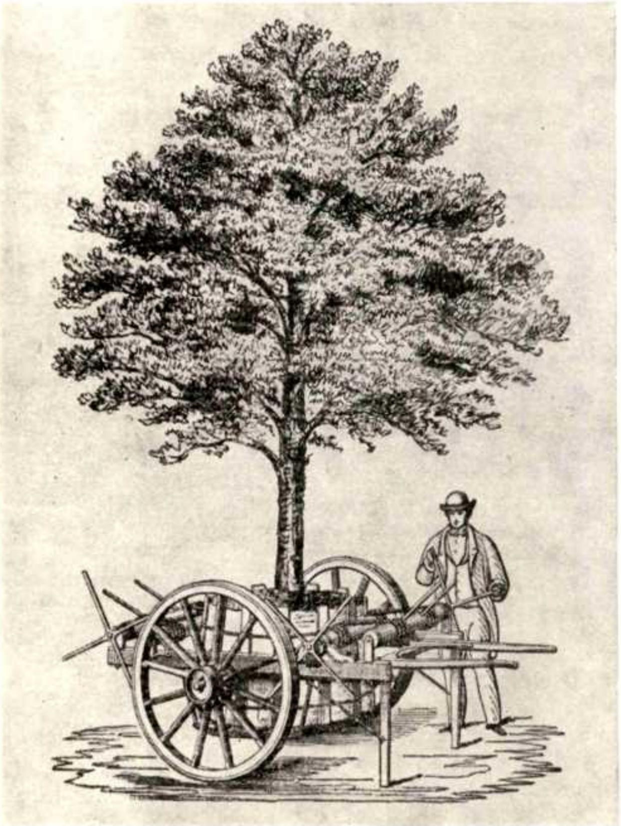


Fig. 2. Tree transplanting machine designed by William McNab and used for transplanting trees from Leith Walk to the present site of the Royal Botanic Garden in the early 1820s.

Graham died in 1845 and was succeeded by John Hutton Balfour who was appointed to the Professorship of Medicine and Botany by Edinburgh Town Council and to the posts of Regius Professor of Botany, Keeper of the Garden and Queen's Botanist by the Crown. Hutton Balfour was a notable teacher whose classes were frequently attended by between 300 and 400 students; provision of practical material for classes of this size must have been a task of considerable proportions—one day in 1873 the number of plant specimens taken from the Botanic Garden to illustrate his lectures and demonstrations was 7,000. For his students Hutton Balfour wrote textbooks almost encyclopaedic in their coverage; one of them was the first British textbook of palaeobotany—his *Introduction to Palaeontological Botany* of 1872. He also continued the emphasis on field work for which the Edinburgh Botany School had been noted for several decades, elaborating the field courses and practising what, at that time, undoubtedly was a fairly advanced ecological botany.

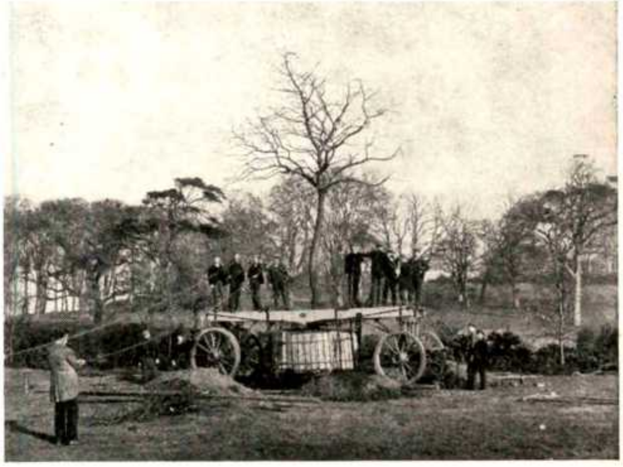


Fig. 3. Tree transplanting machine in operation during the reconstruction which took place at the end of the nineteenth century.

During Hutton Balfour's time as Keeper the fortunes of the Botanic Garden prospered. The acreage was more than trebled, largely due to the incorporation of two adjacent properties. Some of the palms in the tropical palm house, built in Graham's time, had thrived to such an extent that by the early 1850s they were sticking through the roof: their exuberance enabled Balfour to persuade the authorities that a larger palm house must be erected. Parliament voted £6,000 for the project and the new house was completed in 1858. Both palm houses are still in service, although one is 136 and the other 112 years old.

Under Hutton Balfour the Royal Botanic Garden began to assume a form which is recognizable today. He retired in 1880 and was succeeded by one of his students, Alexander Dickson, an able research worker who died after only seven years in office. The next Regius Keeper and Regius Professor of Botany was Hutton Balfour's son, Isaac Bayley Balfour, who held the posts for thirty-four years. With the appointment of Bayley Balfour the modern phase in the history of the Botanic Garden can be said to have begun. He strove tirelessly towards the reconstruction and development of the Botanic Garden and the University Department of Botany in the Garden. The year after his appointment (1889) the entire Garden came wholly under the Crown to be administered by Her Majesty's Office of Works; previously only part of the Garden had been the responsibility of the Crown. The association with the Office of Works, which later became the Ministry of Works and then the Ministry of Public Building and Works, persisted for eighty fruitful years and only came to an end last year.

Aided by a series of brilliant curators (principal gardeners), Bayley Balfour brought about enormous changes: a new range of glasshouses with a frontage of 380 feet was erected, a vast new rock garden was laid out, and extensive rearrangements involving the movement of 40-foot trees were accomplished (Fig. 3). Equally as important, Balfour reorganized the laboratories, provided new accommodation and equipment for botanical research and teaching, and created in Edinburgh a major centre of taxonomic research.

Expeditions and Expansion

The orientation of Bayley Balfour's research and that of most other botanists on the staff of the Botanic Garden during the first half of the twentieth century was brought about by the botanical explorations of George Forrest in Western China. Forrest had held a minor post in the herbarium of the Garden until in 1904 he was induced by Bayley Balfour to undertake the first of his expeditions. As a result of Forrest's introductions the staff of the Garden became particularly interested in Himalayan and West Chinese plants and specialized in such genera as *Rhododendron*, *Magnolia*, *Primula*, *Gentiana* and *Meconopsis*: specializations which are retained today and still strongly influence the work of the scientific staff. The *Notes from the Royal Botanic Garden, Edinburgh*, founded by Bayley Balfour in 1900 as the official scientific publication of the Garden, provided the medium for the communication of the increasing volume of scientific information being produced.

Bayley Balfour was succeeded in 1922 by William Wright Smith who, like both of the Balfours, was Regius Keeper for thirty-four years. During his period of office the Botanic Garden flourished, and the series of developments which have brought it to its present form continued. In 1928 a West Coast Garden of 90 acres came under the control of the Royal Botanic Garden, Edinburgh; it was part of the 10,200 acre estate of Benmore which was gifted to the nation by Harry George Younger of Benmore and Kilmun. This garden, known as the Younger Botanic Garden, was developed as a woodland garden and arboretum on a grand scale, and its provision allowed the successful growing of conifers and other species which had never thrived in Edinburgh. Wright Smith is best remembered scientifically for the description of more than 550 plant species new to science, and the monographic treatment of the genus *Primula* which he carried out in conjunction with Harold Fletcher, the present Regius Keeper. From 1930 to 1954 Wright Smith was supported by John Macqueen Cowan as Deputy Keeper; in Edinburgh, Cowan began an intensive study of the genus *Rhododendron* on which he became the recognized authority.

The years after the Second World War have seen plenty of activity regarding developments in both buildings and staffing. In the late 1940s the civil servants employed in the herbarium and laboratory were incorporated into the new Scientific Civil Service grades recommended by the Barlow Report. The size of the scientific staff was also increased so that the establishment could extend its research output, to bring it more into line with the facilities for investigation offered by the enormous collections of both living and herbarium specimens. In this respect 1951 was an *annus mirabilis* in the history of the Botanic Garden, with five members of the scientific officer grades added to the staff. University staff were also increased so that the department could participate in the explosion of botanical research in the new disciplines.

When Wright Smith died in 1956, the posts of Regius Keeper of the Garden and Professor of Botany in the University had been vested in one person for almost 220 years. Such a system carried with it an inevitable corollary: the Regius Keeper had to be a systematist to carry out the duties involved in directing the Garden, and therefore the Head of the University Botany Department was also a systematist. The arrangement had worked well in earlier times when botany was virtually synonymous with systematic botany, but with the growth of other branches of the science the University felt that it should no longer be restricted to choosing its professor from a single discipline. Accordingly the dual post was split: Harold Fletcher, who had been Deputy Keeper since 1954 when Cowan had retired, was appointed in 1956 Regius Keeper of the Garden, while Robert Brown, well known for his research in cell biology, became Regius Professor of Botany in 1958. Despite this division a close relationship has been maintained between the Royal Botanic Garden and the University Botany Department, and Fletcher was appointed an Honorary Professor of Botany in 1968. He was appointed Queen's Botanist in 1967.

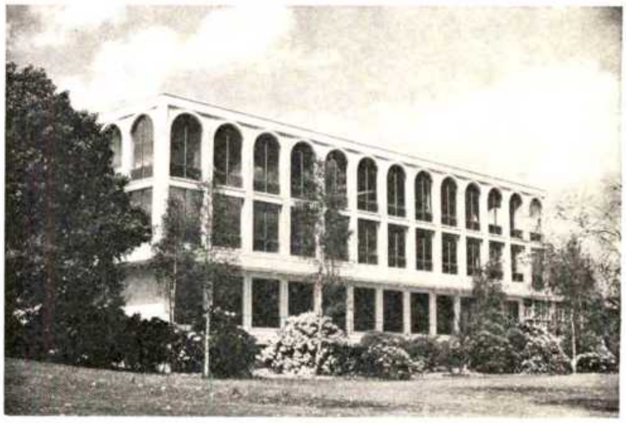


Fig. 4. The Royal Botanic Garden Herbarium, Laboratory and Library building which was completed in 1964.

Too Many Specimens

At the end of the 1950s the accommodation at the Royal Botanic Garden was grossly inadequate. Garden laboratory and administrative staff together with University staff and research students were crowded into a building which had met the spatial requirements of fifty years previously. Conditions in the herbarium were even worse: the collections were housed in an antiquated building, originally built in the early nineteenth century as an exhibition hall, and in two huts which had been erected beside it to take the overspill of specimens. It was obvious that new buildings had to be provided, and the first of these was the new Royal Botanic Garden Herbarium, Library and Laboratory building (Fig. 4), officially opened by Her Majesty the Queen in June 1964. This spacious building, which provides excellent accommodation for the herbarium, library and laboratory of the Garden, was completed just before the Tenth International Botanical Congress was held in Edinburgh in 1964 and its facilities were much admired by the visiting botanists.

The University Botany Department also required new premises and, furthermore, faced problems concerning their correct siting. The Botanic Garden was five miles from the King's Buildings complex in South Edinburgh where the rest of the university science buildings were situated, and its isolation posed considerable problems in the planning of timetables allowing students the necessary travelling time between classes, in intercommunication with other university departments, in access to their libraries, and so on. It was decided, therefore, to break the long tradition whereby university botany teaching had been carried out at the Botanic Garden and to site the new building in the science complex at King's Buildings; in 1965 it was completed. The herbarium collections and library at the Botanic Garden were, however, of vital importance to the university taxonomists, and accordingly their section of the department has remained in a refurbished part of the old building.

An inspired programme of development has also been carried out in the Botanic Garden, directed by the present Curator, Edward Kemp, whose extensive knowledge of landscaping has enabled him to make major improvements in many areas (Fig. 5). A fine new glasshouse of unique design, 420 feet long with a span of 60 feet and a height of 36 feet in its central section, was opened in October 1967 to replace the sixty year old main range which had become dilapidated. This glasshouse is divided into six large compartments, each with a different environment, and its planting has been landscaped to give as natural an effect as possible (Fig. 6).

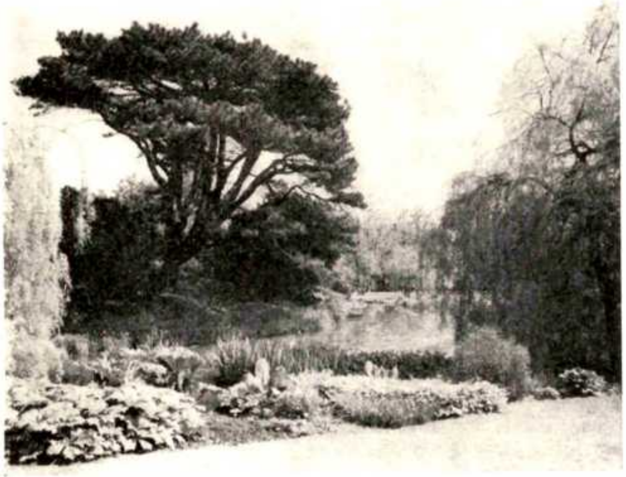


Fig. 5. A view in the present day Botanic Garden showing the pond.

Two other events in the recent history of the Garden should be mentioned here. The first was the transfer of responsibility from the Ministry of Public Building and Works to the Department of Agriculture and Fisheries for Scotland which occurred on April 1, 1969. The second was the acquisition in 1969 of part of the famous Garden of Logan in Wigtownshire, originally created by the brothers McDouall. Thus the Royal Botanic Garden, Edinburgh, now has two important annexes—the Younger Botanic Garden at Benmore and the Logan Botanic Garden.

Research Today

It seems appropriate to conclude this short history with a brief survey of the status at the present day of two of the most important functions of the Royal Botanic Garden: research and education.

The permanent research staff of the institute is relatively small, consisting of fourteen members of the scientific and experimental officer classes, supported by scientific assistants and ancillary grades. A surprisingly high research output is maintained, however, and research projects range from studies of New Guinea Orchids to the preparation of an Agaric flora of the British Isles. University taxonomic staff and research students also play a vital part in the research of the establishment; their leader, Peter Davis, Reader in the Department of Botany, has made numerous expeditions to South-West Asia, particularly Turkey, and his work, like that of Forrest in the days of Bayley Balfour, has greatly influenced the orientation of research in the Royal Botanic Garden. At present Davis's team is engaged in writing the fourth volume of a definitive *Flora of Turkey* which will comprise eight volumes in all.

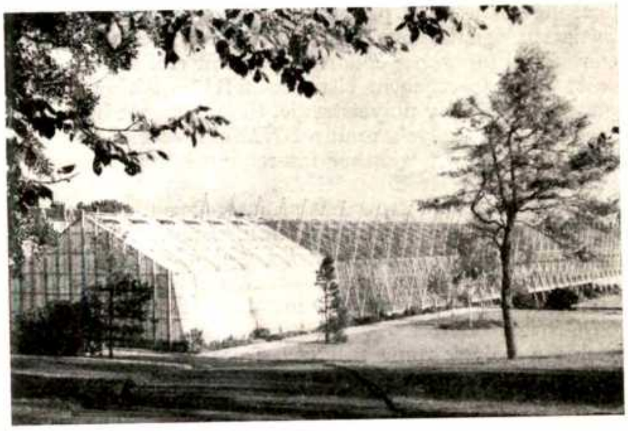


Fig. 6. The principal glasshouse range, 420 feet long, which was completed in 1967. Note the use of external supporting timbers.

Education at various levels is playing an increasingly important part in the work of the Royal Botanic Garden. The three year Horticultural Instruction Course leading to the award of the Diploma in Horticulture (Edinburgh), which has been established for nearly eighty years, continues to flourish and has an annual intake of fifteen students. The instruction in this course is carried out by staff members and also by specialists from other institutes. University and Botanic Garden staff share the teaching of the one-year postgraduate course for the University of Edinburgh Diploma in Plant Taxonomy, while members of the Garden staff who hold honorary lectureships in the University also supervise PhD students. Education of the general public is also acknowledged to be an important function of the Garden, and to further this end a large new demonstration garden which concentrates on the biology of plants was laid out in 1961, and educational sound guides of the new glasshouse range have recently been provided. An anonymous donor was so impressed with the educational potentialities of the Garden that he gave £50,000 for the construction of an Exhibition Hall. This hall, equipped with a series of imaginative exhibits in which the use of audio-vision units plays an integral part, will be officially opened during the tercentenary celebrations.

The Intercistronic Divide: Translation of an Intercistronic Region in the Histidine Operon of *Salmonella typhimurium*

by
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ROBERT G. MARTIN
Laboratory of Molecular Biology,
National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health,
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A frameshift mutation near the end of the *hisD* gene of the histidine operon results in the production of an active *hisD* gene product. Analysis of the C-terminal sequence of the mutant protein establishes that the normal *in vivo hisD* chain termination codon can be UAA or UGA but not UAG, and demonstrates the existence of an intercistronic region between two genes of the operon.

STEITZ has recently demonstrated that the codon AUG is the polypeptide chain initiation signal for each of the three cistrons of the RNA phage R17 (ref. 1). In no case is the initiation signal immediately preceded by a chain termination signal (nonsense codon), so that intercistronic regions exist between adjacent cistrons of R17. Although RNA phages are clearly polycistronic, they may not be suitable models for the polycistronic mRNAs of bacterial operons[1,2]. It is not known whether intercistronic regions exist in bacterial polycistronic mRNAs.

We demonstrate here that an intercistronic region does exist between the second and third structural genes of the histidine operon of *Salmonella typhimurium*. The *his* operon consists of an operator region and nine contiguous structural genes arranged in the order: *operator-hisGDCBHAFIE* (ref. 3). Transcription of the *his* operon yields a polycistronic mRNA[4], from which the second, third and sixth genes (*hisD*, *hisC* and *hisA*) are translated at the same frequency[5]. We describe a frameshift mutation* near the end of *hisD*, *his-2352*, which results in an altered reading frame that allows polypeptide elongation beyond the normal *hisD* termination codon. From the altered *C*-terminal amino-acid sequence of the *hisD* product (histidinol dehydrogenase) in the mutant strain, we deduce that the normal chain termination codon at the end of *hisD* can be UAA or UGA but not UAG; and that an intercistronic region does exist beyond the normal peptide termination signal of *hisD* and contains at least one nucleotide, C, before the next initiation signal. It seems, moreover, that translation of the intercistronic region severely reduces the frequency of translation of the immediately distal cistron.

The *his-2352* mutation was originally selected by Voll[7] in a strain of *S. typhimurium* which contained a histidine operator constitutive mutation, *hisO1242*, and wild type histidine structural genes. Constitutive strains such as *hisO1242* make about ten times as much of the histidine biosynthetic enzymes as does wild type (*S. typhimurium* LT2) (ref. 3) and exhibit the following phenotype: they form wrinkled colonies at 37° C on agar containing 2 per cent glucose, and do not grow at 43° C (ref. 8). By contrast, strains making approximately wild type levels of the distal histidine enzymes (for example, wild type itself, or constitutive strains with a secondary strongly polar mutation) form smooth colonies at either 37° C or 43° C (ref. 8). Voll[7] examined spontaneous revertants of *hisO1242*, selected as smooth colonies growing at 43° C on minimal medium with 2 per cent glucose. Each revertant contained a second mutation in addition to *hisO1242*. One of these, *his-2352*, was mapped (by transduction into a series of overlapping deletions) in the operator-distal part of *hisD* (region VIII), or the proximal part of *hisC* (region I). Enzyme specific activities in *hisO1242 his-2352* relative to *hisO1242* were: *G-enzyme*, 100 per cent; *D-enzyme*, 37–72 per cent; *C-enzyme*, 2 per cent; *B* and *A-enzymes*, 10 per cent. The marked polar reduction of operator-distal gene products could account for smoothness and growth at 43° C. The 2 per cent *C-enzyme* (aminotransferase) activity (corresponding to 20 per cent of the level in wild type cells grown either in minimal media or with excess histidine) allowed growth in minimal media at a nearly normal rate.

It was not known whether the *his-2352* mutation was in *hisD* or *hisC*. On repeated examination, *D-enzyme* (histidinol dehydrogenase) activity in *hisO1242 his-2352* was 65 per cent (± 10 per cent) of that in *hisO1242*. Cell-free extracts of the two strains were examined by acrylamide gel electrophoresis (Fig. 1). The electrophoretic mobilities of wild type histidinol dehydrogenase (from *hisO1242*) and the enzyme from *hisO1242 his-2352* are clearly different. The simplest interpretation is that the primary structure of the histidinol dehydrogenase is altered in *hisO1242 his-2352*. The mutation has therefore been designated *hisD2352*.

Unusual Properties of *hisD2352*

Merely placing the mutated site in *hisD2352* in *hisD*-region VIII, however, does not explain its special properties. First, *hisO1242 hisD2352* is unusually polar, making no more than 10 per cent of the distal enzymes (relative to *hisO1242*). By contrast, five other polar mutations in *hisD*-region VIII are only weakly polar, making ~50 per cent of the distal enzymes (unpublished results of C. B. Bruni and M. M. R.). The strong polarity of *hisD2352* resembles more closely that of mutations at the beginning of *hisC*, which make approximately 10 per cent of the later gene products[13,14]. Second, *hisD2352* is unusual in that it affects more profoundly the expression of the immediately succeeding gene, *hisC* (2 per cent of the constitutive level), than of the later cistrons, *hisB*, *hisH*, and so on (10 per cent of the constitutive level). Instances of non-coordinate polarity in other bacterial operons have been explained by the existence of internal promoters[2], but the extremely weak promoter at *hisB* cannot account for this difference[15]. Because the wild type aminotransferase is fully active in mixed extracts with the aminotransferase from *hisO1242 hisD2352*, the low activity cannot be attributed to an enzyme inhibitor. The specific reduction of aminotransferase activity in *hisO1242 hisD2352* thus seems to be a direct consequence of the *hisD* mutation.

We have considered several mechanisms by which *hisD2352* could produce a structurally altered histidinol dehydrogenase, a coordinate 10 per cent polarity for *hisB*, *hisH* and so on, and a special decrease in aminotransferase activity. (1) A frameshift mutation in *hisD* might allow translation of the dehydrogenase protein beyond the normal termination signal, until a new chain termination codon is reached in the intercistronic region or in *hisC*. Reinitiation of protein synthesis might occur either at 10 per cent of the normal frequency within *hisC* (the aminotransferase would have a reduced molecular activity—specific activity of the pure enzyme[16]—because it lacked part of its normal N-terminal end), or at 2 per cent of normal frequency at the normal *hisC* initiation triplet or within *hisC* (without reduction in the aminotransferase molecular activity) and an additional 8 per cent of normal frequency at the beginning of *hisB*. Alternatively, (2) *hisD2352* might contain an out-of-phase deletion of part of the *hisD–hisC* intercistronic divide, including all or part of the normal termination codon at the end of *hisD*. Reinitiation of protein synthesis would occur as in (1). Or (3) separate point mutations in *hisD* and *hisC* might be present. To distinguish these possibilities, we attempted to relieve the polarity of *hisO1242 hisD2352* by further mutation.

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* A frameshift mutation is one which results from the addition or deletion of one or a limited number (but not three or a multiple of three) of bases from the DNA[6].

HisD2352 is a Frameshift Mutation

Analysis of non-polar constitutive revertants of *hisO1242 his D2352* indicates that *hisD2352* is a frameshift mutation. These mutants were selected as aminotriazole-resistant wrinkled colonies on minimal agar containing 2 per cent glucose. Aminotriazole is an inhibitor of the *B-enzyme*[17]. At appropriate concentrations it inhibits the growth of the prototrophic polar *hisO1242 hisD2352* but not *hisO1242* (which lacks the polar secondary mutation and has tenfold higher levels of the *B-enzyme*). Wrinkled aminotriazole-resistant non-polar revertants were induced by ICR-191, an acridine half-mustard highly specific for inducing frameshift mutations[18], but not by N-methyl-N′-nitro-N-nitrosoguanidine, primarily a base-substituting agent. The enzyme profiles of the revertant strains are shown in Table 1. The *G*, *C* and *B-enzyme* activities were equal to those of *hisO1242* (± 1 standard deviation); that is, polarity was completely relieved as expected. Histidinol dehydrogenase (*D-enzyme*) activity, by contrast, was further reduced below *hisO1242 his D2352* levels, to 10–25 per cent of the *hisO1242* value. The complete restoration of aminotransferase activity with additional loss of histidinol dehydrogenase activity makes it extremely unlikely that *hisD2352* contains separate point mutations in *hisD* and *hisC* (for two corrective mutations would be required) or a deletion of the intercistronic region and part of *hisC*. We conclude that *hisD2352* is a frameshift mutation near the end of *hisD* and propose that the wrinkled revertants contain a second frameshift mutation which corrects the reading frame error of *hisD2352* before the normal *hisD* termination signal. The presence of both frameshift mutations in the revertants further reduces the specific activity of the histidinol dehydrogenase below that of *hisD2352*.

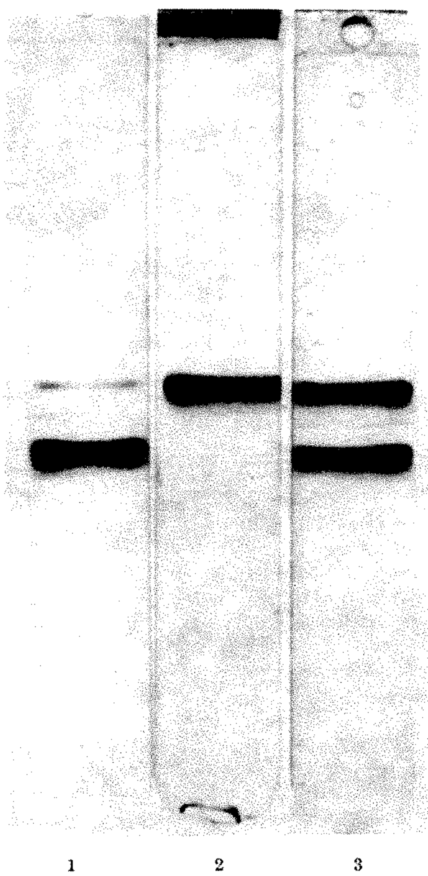

Fig. 1. Acrylamide gel electrophoresis of extracts of *hisO1242* and *hisO1242 his-2352*. Bacterial strains were grown to late logarithmic phase at 37° C with aeration in 200 ml. of minimal medium E[9] supplemented with 0·5 per cent glucose. Cells were harvested by centrifugation, resuspended in 2·5 ml. of 0·05 M Tris-HCl (*p*H 7·5), and disrupted in a French pressure cell at 10,000 lb inch^{-2}. The extracts were clarified by centrifugation at 27,000*g* for 25 min. The supernatant fractions were passed through a 9 × 1 cm column of 'Sephadex G-50', equilibrated with the same Tris-HCl buffer, and the excluded fractions which contained the protein were collected. Extracts were assayed for histidinol dehydrogenase[10]. Disc gel electrophoresis was performed at *p*H 9·5 according to Davis[11]. (1) *hisO1242*, (2) *hisO1242 his-2352*, (3) both. Samples (250 ul.) with 0·05 units of dehydrogenase in Tris-HCl containing 10 per cent sucrose were layered above the spacer gel. The dehydrogenase migrated downward toward the anode. The lower gel margin corresponds to the bromphenol blue front. Gels were stained directly for histidinol-specific dehydrogenase by reduction of a tetrazolium dye (ref. 12, omitting gelatine). The faint upper band in sample (1), also present in (2) and (3), is observed even without adding histidinol, and probably represents a dehydrogenase with different substrate specificity. The distinct electrophoretic mobilities of the wild type and *his-2352* histidinol dehydrogenases are also observed when these strains are grown in the presence of excess histidine, in a non-constitutive genetic background, and when both fully purified enzymes are compared.

Table 1. ENZYME LEVELS IN REVERTANTS OF *hisO1242 hisD2352*

Strains	Enzyme specific activity Synthetase *G*	Dehydrogenase *D*	Aminotransferase *C*	Phosphatase *B*
hisO1242	1·00	1·00	1·00	1·00
hisO1242 hisD2352	0·90	0·73	0·02	0·08
TM217	0·93	0·14	0·81	0·87
TM218	0·98	0·22	0·97	1·00
TM219	1·14	0·19	1·02	1·03
TM220	0·95	0·11	0·86	0·94

The lower four strains (TM217–220) are independently isolated wrinkled revertants obtained from *hisO1242 hisD2352*. They were isolated from minimal plates containing 2 per cent glucose, 5–15 mM 3-amino-1,2,4-triazole, a competitive inhibitor of imidazole glycerol phosphate dehydrase (*B-enzyme*) to suppress background growth[17], and 0·4 mM adenine (to overcome an aminotriazole-induced defect in purine biosynthesis). Approximately 2 × 10^{6} bacteria were spread on each of sixty plates. One-half of the plates received crystals of NG, the other half crystals of 'ICR–191' (a gift of Dr Hugh J. Creech). TM218–220 were induced by 'ICR–191', no wrinkled revertants with non-polar phosphatase levels were induced by N-methyl-N′-nitro-N-nitrosoguanidine. TM217 probably arose spontaneously, for it was located in the periphery of the plate, well outside the ring of mutants induced by 'ICR–191'. Cell-free extracts were prepared as described in the legend to Fig. 1, except that media were supplemented with 0·1 mM L-histidine. Phosphoribosyl-ATP synthetase (*G-enzyme*) (ref. 19 as modified by H. J. Whitfield, jun., manuscript in preparation), histidinol dehydrogenase (*D-enzyme*)[12], histidinol phosphate aminotransferase (*C-enzyme*)[20], and histidinol phosphate phosphatase (*B-enzyme*)[21], were assayed as described previously. Protein was estimated by the method of Lowry *et al.*[22] Enzyme specific activities are given relative to *hisO1242*. All assays were performed at least in duplicate. The average phosphatase level for *hisO1242 hisD2352* in many different experiments was 0·10 rather than 0·08 as observed in this experiment. Aminotransferase values never exceeded 0·02.

HisD2352 differs from other frameshift mutations in *hisD*-region VIII in that it is strongly polar and preferentially affects aminotransferase activity. Both effects may be explained by the translation of *hisD* beyond its normal termination signal. The frameshift resulting from the addition or deletion of bases in *hisD2352* leads to new codon groupings specifying the addition of different amino-acids to the carboxy end of histidinol dehydrogenase, until a chain termination signal in the new reading frame is reached. We have found that the first new nonsense triplet arises beyond the normal *hisD* termination codon.

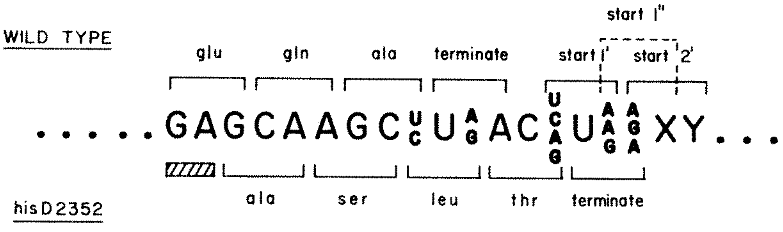

Fig. 2. Sequence of the mRNA corresponding to the end of *hisD* and the start of the intercistronic region. The deletion of two bases (GA) indicated by the hatched area most simply generates the sequence of the four C-terminal amino-acids in the mutant dehydrogenase. The same reading-frame and amino-acid sequence could, however, arise by adding one base to the first GA shown to generate an additional arginine (AGA, CGA) or glycine (CGA) codon. Glycine has been recovered in varying amounts after acid hydrolysis of peptide R eluted from paper; it is not clear if this comes entirely from the paper blank. Arginine, if present, would be released as the free amino-acid on tryptic digestion, but would not be detected on peptide mapping because the wild type dehydrogenase already has a free arginine spot. Whether the mutational event in *hisD2352* is a +1 addition or −2 deletion does not affect the sequence analysis of the C-terminal region of the enzyme or of the intercistronic space. The overlapping sequences uniquely define all amino-acid codons as shown and the normal termination signal as UNA, but do not eliminate the possibility that the termination codon is either UUA or UCA.

This implies that the nucleotides specifying the normal C-terminal amino-acid(s) of histidinol dehydrogenase, as well as the *hisD* termination codon itself, and at least part of the intercistronic space are translated out-of-phase in *hisD2352* as the C-terminal portion of its histidinol dehydrogenase.

The Intercistronic Region

To delineate the normal intercistronic region, we analysed the C-terminal sequence of histidinol dehydrogenase from *hisO1242 hisD2352*. Yourno previously reported the purification[23] and tryptic peptide map[24] of the wild type enzyme from *hisO1242*. He identified the C-terminal tripeptide, T18, and established its sequence as Glu-Gln-Ala[24] (J. Yourno, personal communication). We have purified the dehydrogenase from *hisO1242* and *hisO1242 hisD2352* by analogous procedures. Tryptic peptide maps[25] of the mutant dehydrogenase show the unique disappearance of the wild type peptide, T18, and the appearance of a single new peptide, R. On acid hydrolysis, peptide R yields equimolar amounts of alanine, threonine, leucine and serine. Carboxypeptidase A digestion[26] of histidinol dehydrogenase from *hisD2352* leads to the rapid release of threonine and leucine, and the slower release of serine and alanine with a strong suggestion that the order is Ala-Ser-(Leu,Thr). By hydrazinolysis[27] threonine was identified as the C-terminal amino-acid. Thus, the C-terminal sequence of wild type histidinol dehydrogenase, Glu-Gln-Ala, has been changed in *hisD2352* to Ala-Ser-Leu-Thr. The reduction of overall negative charge that results from the loss of a glutamic acid residue is consistent with the slower electrophoretic mobility toward the anode of the mutant enzyme (Fig. 1).

Using the amino-acid sequences of the C-terminal ends of wild type and mutant dehydrogenases, and the known codon assignments, the nucleotide sequence of the mRNA of this region can be deduced. The C-terminal sequence of the enzyme synthesized in *hisO1242 hisD2352* can be generated from the wild type sequence by a + reading-frame shift[6], that is, from a two-base deletion or a one-base addition (Fig. 2). The finding that -Leu-Thr is the C-terminal sequence in the mutant dehydrogenase indicates that the normal *hisD* terminator codon must be UNA (presumably one of the nonsense codons, UAA or UGA); a third nonsense codon, UAG, is excluded. The possibility that the normal *hisD* termination signal contains two adjacent nonsense codons (as in bacteriophage R17) (ref. 28) is also eliminated.

The presence of the nucleotide C, following the *hisD* termination signal, would establish the existence of an intercistronic region before the initiation codon of *hisC*, provided that codon did not begin with the nucleotide C. Probably the aminotransferase is synthesized, as are other bacterial and bacteriophage proteins, with N-terminal N-formylmethionine (coded by AUG or GUG)[29]. Analysis of the purified aminotransferase reveals serine as the only N-terminal amino-acid. (In addition to the identification of DNP-serine by the fluorodinitrophenol method[30], we have now identified N-terminal serine both by the dansyl chloride[31] and acetic anhydride acetylation[32] reactions. Quantitation of N-terminal amino-acids by the method of Stark and Smyth[33] revealed 0·7 serine equivalents and less than 0·2 equivalents of all other amino-acids.) N-Terminal serine might arise after cleavage of formylmethionine[29] or truly be the initial amino-acid. Because no codon for formylmethionine or serine begins with C, this nucleotide must be part of the intercistronic space.

If serine were the *hisC* initiation signal, the earliest possible initiator codon would be 1″ in Fig. 2; that is, the intercistronic region would consist of three nucleotides, CNU. In the more likely event that a formylmethionine codon is the aminotransferase initiator, the earliest initiation could occur at codon 1′ (leaving the single nucleotide, C, as the intercistronic space) or at 2′ (separated by the four nucleotides, CNUPu, from the *hisD* termination signal). Alternatively, the intercistronic space might be still longer.

The sequence analysis establishes that there is normally an intercistronic space, and that at least part of it is translated as a result of the *hisD2352* mutation. It is not clear, however, how translation of five additional nucleotides (the normal *hisD* termination codon and two succeeding nucleotides) inhibits the expression of *hisC* in *hisO1242 hisD2352*. The preferential inhibition of *hisC* activity can be explained either by assuming that *hisC* is translated less frequently than *hisB*, or that it is translated at the same frequency but yields an aminotransferase with a reduced molecular activity. The following experiments argue against the latter possibility. Extracts from *hisO1242* and *hisO1242 hisD2352* give a reaction of identity in Ouchterlony double diffusion experiments with absorbed antiserum[14] against purified wild-type aminotransferase. This suggests either that the aminotransferase is identical in both strains or that the antiserum contains no major component directed against the N-terminal end of the aminotransferase. In quantitative experiments, a constant amount of antiserum neutralizes the same number of units of aminotransferase activity in extracts of *hisO1242* and *hisO1242 hisD2352*, supporting the conclusion that the aminotransferase has identical molecular activity in the two strains. Otherwise, the data require that in extracts of *hisO1242 hisD2352* both the reactivity with antiserum and the activity of the aminotransferase decrease to precisely the same extent, from 10 per cent of *hisO1242* levels (that found for *hisB*, *hisH* and so on, in *hisO1242 hisD2352*) to 2 per cent of *hisO1242* levels. Not only is this parallel loss most unlikely, but it clearly conflicts with the result of the double diffusion experiments; the aminotransferase in the mutant strain has not lost a major antigenic determinant. Thus the aminotransferase in *hisO1242 his D2352* has the same molecular activity as that found in *hisO1242*.

The reduced aminotransferase activity in *hisO1242 hisD2352* must therefore result from a low frequency of initiation at the normal *hisC* initiator or at a new internal initiation site within *hisC*. In either case, initiation at the normal *hisC* initiator must be reduced or abolished in *hisO1242 hisD2352*. If the wild type *hisC* initiation signal is either codon 1′, 1″, or 2′ (Fig. 2), then the same nucleotide(s) in *hisO1242 hisD2352* must specify both termination of *hisD* and initiation of *hisC*, and be recognized by both polypeptide termination factors and initiation factors[29]. This overlap might reduce the efficiency of initiation at *hisC* but allow synthesis of a completely normal aminotransferase. This interpretation is analogous to the proposal[34] that following premature polypeptide chain termination in certain mutants of bacteriophage T4, ribosomes may phaselessly wander back to an earlier polypeptide starter signal.

On the other hand, if none of the start signals shown in Fig. 2 is the normal *hisC* initiator, then the wild type intercistronic space must consist of more than five nucleotides. Translation of the *hisD2352* dehydrogenase would terminate entirely within the intercistronic space. Although the nucleotide signals for termination of *hisD* and initiation of *hisC* would not overlap, some steric interference of polypeptide termination factors with initiation factors or terminating ribosomes with initiating ribosomes might still occur. Alternatively, translation of the intercistronic space might disrupt local mRNA secondary structure and thereby interfere with initiation at *hisC*. Whatever the mechanism, it seems that the intercistronic divide is vital in controlling the initiation of protein synthesis in the internal cistrons of a polycistronic message.

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Embryopathic Effects of 7,12-Dimethylbenz(a)anthracene and its Hydroxymethyl Derivatives in the Sprague–Dawley Rat

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7,12-Dimethylbenz(a)anthracene and one of its hydroxymethyl derivatives have been shown to cause a variety of foetal abnormalities, especially when administered on day 13 of pregnancy. It is suggested that derivatives of benz(a)anthracene must have active side chains situated at C-7 and C-12 for embryopathic activity.

THE polycyclic carcinogenic hydrocarbon, 7,12-dimethylbenz(a)anthracene (DMBA), produces selective necrosis of the adrenal cortex in the mature rat[1]. The main products of the metabolism of DMBA by rat liver homogenates are the isomeric monohydroxymethyl derivatives, 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-12-MBA) and 12-hydroxymethyl-7-methylbenz(a)anthracene (12-OHM-7-MBA)[2]; 7-OHM-12-MBA is a more potent adrenocorticolytic agent than DMBA[3,4], whereas 12-OHM-7-MBA has no adrenocorticolytic effect[3]. What follows is a preliminary report of investigations of the effects of DMBA and its monohydroxymethyl derivatives on the pregnant rat and rat foetus.

Female non-parous Sprague–Dawley rats, 220–300 g in weight, were caged with males, and the day on which spermatozoa were found in the vaginal smear was designated day 0 of pregnancy. When rats were treated after implantation, laparotomies were performed before treatment to confirm pregnancy and to determine the number of implantation sites. As a routine practice the rats were killed with chloroform on day 20 of pregnancy, the day before expected delivery. All foetuses were examined macroscopically, measured and weighed, and representative examples necropsied or studied by freehand razor sections. At least two foetuses from each litter were fixed in 95 per cent ethanol and the skeletal tissues stained with alizarin red. All placentae were weighed, and several from each mother were retained for histological examination. The maternal adrenals and ovaries were also studied histologically.

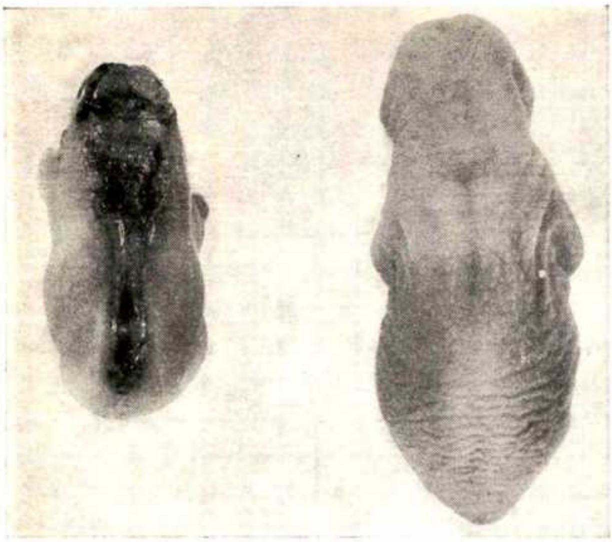

Fig. 1. Foetus from a litter of a rat treated with 7-OHM-12-MBA on day 13 of pregnancy and killed on day 20. Note the posterior encephalocele, the spina bifida extending to the lumbar region, and the marked stunting. Right, control foetus (× c. 1·5).

Effects of 7-OHM-12-MBA

7-OHM-12-MBA was dissolved in olive oil and emulsified with saline, giving a final concentration of 3 mg 7-OHM-12-MBA per ml. of 30 per cent oil emulsion. Control rats received an equivalent volume of emulsion containing no hydrocarbon.

Table 1. EMBRYOPATHIC EFFECTS OF 7-OHM-12-MBA (2·5 mg/100 g MATERNAL BODY WEIGHT) IN THE SPRAGUE–DAWLEY RAT

Days pregnant when treated with 7-OHM-12-MBA	No. rats treated	Total No. of implantations	No. resorptions* (per cent)	No. surviving foetuses with malformations
2	4	45	3 (7)	0
3	6	63	31 (49)	0
4	10	137	107 (78)	0
5	6	76	38 (50)	0
6	8	85	35 (41)	0
7	11	128	76 (59)	0
8	8	88	88 (100)	—
9	8	111	85 (77)	0
10	10	129	89 (69)	0
11	14	152	23 (15)	129†
12	8	101	10 (10)	91†
13	9	95	15 (16)	80†
14	10	129	9 (7)	120†
15	10	131	12 (9)	119†
16	13	144	44 (31)	0
17	8	88	9 (10)	0
18	4	51	1 (2)	0

* Resorption rate in control groups: mean 7 per cent; range 2–29 per cent.
† Note 100 per cent of surviving foetuses have malformations.

In the first experiment rats were treated with a uniform dose of 7-OHM-12-MBA, each rat receiving one single intravenous injection at a dose of 2·5 mg/100 g body weight; this was investigated from day 2 to day 18 of pregnancy (Table 1). 7-OHM-12-MBA administered between day 3 and day 10 of pregnancy increased the resorption rate and this reached a maximum (100 per cent) on day 8; no gross skeletal or other malformations were found in any of the survivors. In the control series an average of 7 per cent of embryos resorbed between implantation and term, although in a few groups a resorption rate of up to 29 per cent was found. Treatment between day 11 and day 15 of pregnancy caused teratogenic effects, but the resportion rate was within normal limits (Table 1).

Treatment on day 12 to day 14 affected every foetus in each litter, each foetus showing almost identical pathology to its littermates: stunting, lordosis of cervical and upper thoracic parts of the vertebral column, and an encephalocele and a spina bifida (Fig. 1). They all seemed to be oedematous: this was caused by a layer of gelatinous material in the deep part of the dermis and in the underlying subcutaneous tissues.

The encephalocele, which was covered with an attenuated layer of skin, was about the same size in each foetus and involved the posterior part of the skull. The opening in the skull, through which the posterior parts of the brain protruded, was caused by partial or complete failure in the development of the interparietal and occipital bones (Fig. 2). The spina bifida and the associated meningomyelocele varied in size according to the day of treatment with 7-OHM-12-MBA. The most severe lesion followed treatment on day 13, when the defect involved almost the entire length of the vertebral column (Fig. 1); with treatment on day 12 the defect extended to the thoracic region, while the treatment on day 14 produced a lesion that involved only the cervical region. There was no obvious histological abnormality of the brain or of the spinal cord. The vertebral arches in the affected parts of the spine were severely stunted and the remnants were everted. The alizarin red preparations revealed other skeletal malformations, which were also most severe after treatment on day 13 (Fig. 2). These were partial or complete failure of development of the supraspinous portion of the scapula, complete inhibition or severe retardation of rib development, and shortening of some of the digits of the forepaws. Treatment on day 11 or day 15 of pregnancy produced similar but less severe teratogenic effects.

No malformations of the axial skeleton or other bony abnormalities, except for one foetus with a hypoplastic mandible, have been found in about 2,000 control foetuses.

A significant reduction in placental weight occurred during the 11 to 14-day treatment period and was most marked with treatment on day 12; this was associated with a proportionate reduction in foetal size.

Histologically, the placentae showed a relatively poor development of the syncytiotrophoblast; the cytotrophoblast, which contained cystic areas, showed an increase in nuclear pleomorphism and more extensive necrosis than in controls. The ovaries showed no histological abnormality.

The adrenal glands of every mother treated with 7-OHM-12-MBA were necrotic and the adrenals of their foetuses showed interesting histopathological changes: for example, in the litters treated on day 17, there was extensive necrosis of the central parts of the glands[4a].

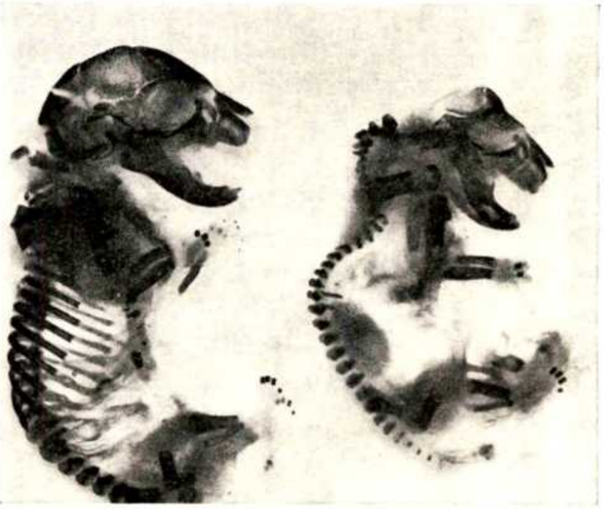

Fig. 2. Right, foetus from a litter of a rat treated with 7-OHM-12-MBA on day 13 of pregnancy. Note the absence of the interparietal and occipital skull bones, the incomplete development of the scapula, the almost complete absence of ribs and the severe cervico-thoracic lordosis. Left, control foetus (alizarin red, × c. 1·7).

Table 2. EFFECT OF VARYING THE DOSE OF 7-OHM-12-MBA ON THE EMBRYOPATHIC EFFECTS PRODUCED WITH TREATMENT AT DAY 8 OF PREGNANCY

7-OHM-12-MBA (mg/100 g maternal body weight)	No. rats treated	No. rats surviving to day 20	Total No. implantations	No. resorptions (per cent)*	No. surviving foetuses with malformations
0·1	3	3	41	1 (2)	0
0·5	3	3	46	4 (9)	0
1·0	2	2	29	5 (17)	10
1·5	3	3	42	10 (24)	14
2·0	3	3	40	22 (55)	5
2·5	3	3	30	30 (100)	—

* Resorption rate in control groups: mean 5 per cent.

The results of varying the dose of 7-OHM-12-MBA at day 8 of pregnancy are shown in Table 2. The resorption rate increased in proportion to the dose of 7-OHM-12-MBA and, as already indicated, it was 100 per cent with a dose of 2·5 mg/100 g maternal body weight. With doses of between 1·0 mg and 2·0 mg/100 g maternal body weight, teratogenic effects were produced in some of the surviving foetuses. The malformations had no special predilection for the axial skeleton, and included facial cleft, cleft lip, cleft palate, eventration of abdominal viscera, encephalocele, microphthalmia, cleft mandible, hypoplasia of mandible and maxilla, talipes, syndactyly, agenesis of kidney, cervical spina bifida, short tail, fused ribs and fused cervical vertebral arches. There was a reduction in the foetal and placental weights and this was more or less in inverse proportion to the dose of 7-OHM-12-MBA.

Varying the dose of 7-OHM-12-MBA at day 13 of pregnancy showed that a dose of 0·1 mg or 0·5 mg/100 g maternal body weight did not increase the resorption rate or produce teratogenic effects. With a dose of 1·0 mg/100 g maternal body weight or greater, however, all of the foetuses surviving to day 20 of pregnancy had axial skeleton malformations of the type described above. The encephalocele was of constant appearance, but the extent of the spina bifida varied with the dose of 7-OHM-12-MBA: 1·0 mg–2·0 mg/100 g maternal body weight produced a spina bifida involving the cervical and thoracic parts of the vertebral column whereas 2·5 mg–3·5 mg/100 g maternal body weight caused a lesion extending to the lumbar segments. The weights of foetuses and placentae were reduced and, in general, there was a direct relationship between dose and effect. The resorption rate was not significantly increased except with doses of 3 mg/100 g maternal body weight or greater; a dose of 4 mg/100 g body weight was extremely toxic for the mothers, and none survived to day 20.

There was a fairly close correlation between the dose of 7-OHM-12-MBA, the degree of damage of the maternal adrenal cortex, and the extent of the teratogenic effects.

Effects of DMBA, 12-OHM-7-MBA and 7-OHMBA

The effects of administering DMBA, 12-OHM-7-MBA, and a related benz(a)anthracene derivative, 7-hydroxymethylbenz(a)anthracene (7-OHMBA), on day 8 or day 13 of pregnancy, were studied. A single intravenous injection of an emulsion of DMBA or 12-OHM-7-MBA (2·5 mg/100 g maternal body weight), or a single intraperitoneal injection of 7-OHMBA (2·5 mg or 3·5 mg/100 g maternal body weight), dissolved in olive oil, was given. Control groups received an equivalent volume of emulsion or olive oil without the hydrocarbon.

The results are summarized in Table 3.

12-OHM-7-MBA and 7-OHMBA did not significantly increase the resorption rate or produce teratogenic effects on day 8 or day 13 of pregnancy. In contrast, treatment with DMBA, 2·5 mg/100 g maternal body weight, on day 8 of pregnancy increased the resorption rate, but not to the same extent as the equivalent dose of 7-OHM-12-MBA. A proportion of the surviving foetuses had malformations; the range and type were similar to that found with low doses of 7-OHM-12-MBA on day 8 of pregnancy.

DMBA treatment on day 13 of pregnancy did not increase the resorption rate, but teratogenic effects were produced and the malformations were similar to, but less severe than, those produced by 7-OHM-12-MBA on day 13. Over half of the surviving foetuses had a small posterior encephalocele, but there was no obvious spina bifida in any of them. Alizarin red preparations, however, revealed that whether or not there was an encephalocele, in every foetus the interparietal and occipital bones of the skull were incompletely developed and in some cases totally absent. Some foetuses showed eversion of the arches of the upper cervical vertebrae, and incomplete development of the supraspinous wing of the scapulae and the ribs. A dose of 2·5 mg/100 g of DMBA was highly toxic to the mothers (Table 3). Treatment with DMBA on day 8 or day 13 of pregnancy induced massive necrosis of the inner zones of the cortex of all the maternal adrenal glands.

Table 3. EMBRYOPATHIC EFFECTS OF THE DIMETHYL AND HYDROXYMETHYL DERIVATIVES OF BENZ(A)ANTHRACENE FOLLOWING TREATMENT AT DAY 8 OR DAY 13 OF PREGNANCY

Benz(a)anthracene derivative	No. rats treated	No. rats surviving to day 20	Total No. implantations in surviving rats	No. resorptions (per cent)*	No. surviving foetuses with malformations
Day 8 treatment					
DMBA	9	5	64	39 (61)	7
12-OHM-7-MBA	5	5	63	10 (16)	0
7-OHMBA	4	4	46	12 (26)	0
Day 13 treatment					
DMBA	11	4	46	4 (9)	42†
12-OHM-7-MBA	5	5	63	7 (11)	0
7-OHMBA	4	4	55	3 (5)	0

* Resorption rate in control groups: mean 7 per cent.

† Note 100 per cent of surviving foetuses have malformations.

The effects of DMBA and 7-OHM-12-MBA in the pregnant Sprague–Dawley rat at day 8 or day 13 of pregnancy were similar in many respects, but clearly 7-OHM-12-MBA was the more potent embryopathic agent. Because DMBA and 7-OHM-12-MBA are embryopathic whereas 12-OHM-7-MBA and 7-OHMBA are not, it suggests that for embryopathic activity, as for adrenocorticolytic activity[5], derivatives of benz(a)anthracene must possess two active side chains situated at C-7 and C-12, with an intact methyl group at C-12 being mandatory, and at C-7 one of several possible substituents of which a hydroxymethyl group seems to confer the most potent activity.

With few exceptions the site and mode of action of embryopathic substances are largely unknown: they might have a direct toxic effect on differentiating tissues at the time of organogenesis, or affect the maternal environment or the placenta, or act by a combination of these factors. The mechanism of action of DMBA and 7-OHM-12-MBA in producing their embryopathic effects is as yet unknown.

DMBA and 7-OHM-12-MBA are potent adrenocorticolytic agents in the adult Sprague–Dawley rat, and all the pregnant rats treated with these compounds showed severe adrenocortical necrosis. There is a rapid fall in adrenal and plasma corticosterone levels beginning 24 h after treatment of rats with DMBA[6,7], and it is almost certain that 7-OHM-12-MBA has similar effects. It seems possible that such marked alterations in maternal adrenocorticosteroid levels, occurring at a critical stage of development, could affect the embryo. Adrenalectomy, however, in the pregnant rat does not cause teratogenic effects although there are conflicting reports as to whether it is associated with an increase in the resorption rate[8–12]. It is unlikely that the teratogenic effects reported here are directly caused by the maternal adrenal necrosis.

It has been reported that ovariectomized rats and mice deficient in progesterone produce congenitally abnormal foetuses[13,14], but in our experiments the ovaries of the DMBA- and 7-OHM-12-MBA-treated animals showed no histological abnormality.

Certain teratogenic factors are known to affect placental function[15,16]. In our experiments with 7-OHM-12-MBA and DMBA the placenta was reduced in size and showed histological changes, and it is possible that a disturbance in placental function may play some part in the production of the embryopathic effects.

A number of embryopathic substances are known to cross the placental barrier in rodents[17,18], and some of these may have direct toxic effects on developing embryonic tissues. Cytotoxic agents are thought to inhibit cellular proliferation in a non-specific fashion at critical stages of embryogenesis[19], but salicylates are believed to depress specifically the biosynthesis of mucopolysaccharides[20]. DMBA depresses the synthesis of DNA in certain rat tissues[21] and it is possible that 7-OHM-12-MBA may have similar effects.

It seems that the ultimate embryopathic substance may be a metabolite of 7-OHM-12-MBA because we have shown that the embryotoxic and teratogenic effects of 7-OHM-12-MBA at day 8 and day 13 of pregnancy respectively are inhibited by pretreatment with 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (Metopirone, Ciba; Su 4885) or with β-diethylaminoethyldiphenyl-n-propyl acetate (SKF 525-A) (unpublished results). It is not known if the ultimate adrenocorticolytic and embryopathic agents are different derivatives of 7-OHM-12-MBA or where they are metabolized.

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Early Pliocene Sediments and Fossils from the Northern Kenya Rift Valley

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Mammalian fossils, which include the tooth of an early hominid, have been recovered from the newly mapped Ngorora Formation in the Baringo District. Preliminary dating suggests that this formation is aged between 9 and 12 million years, and the new finds thus help to span a ten million year gap in the fossil mammal record in Africa south of the Sahara.

IN 1967, preliminary details were published of the discovery of mammalian fossils, including a hominid temporal, from the Kenya Rift Valley near Lake Baringo[1]. Geological investigation of the Baringo District has continued as part of a long term mapping project undertaken by the East African Geological Research Unit (EAGRU) based at Bedford College, University of London, and under the direction of Professor B. C. King.

In February 1968 in the course of the EAGRU mapping programme, one of us (G. R. C.) located a sedimentary formation from which some well preserved mammalian fossils, including the crown of a hominid molar, were recovered. The sediments outcrop in the Ngorora administrative location some twenty miles north-west of Lake Baringo (Fig. 1). Further, more detailed, stratigraphical and palaeontological investigations were undertaken during July and August of 1968 and 1969. The fossiliferous sediments are underlain and overlain by flows of phonolite lava for which preliminary potassium–argon dates have been obtained. These suggest that the sediments are aged between 9 and 12 m.y. Preliminary descriptions of the sediments and fossils follow, and the fossiliferous sequence is named the Ngorora Formation.

Ngorora Formation

This unit consists typically of well bedded, waterlaid fossiliferous sediments showing great variation in both thickness and lithology over the relatively limited extent of the outcrop. Lithologies are dominated by volcanic material, but range over a complete pyroclastic-epiclastic spectrum from primary pumice-tuffs and agglomerates to arenites and conglomerates composed chiefly of well rounded lava and feldspar clasts. Secondary zeolites are common in the coarser lithologies.

The Type Area

The maximum observed thickness occurs in the Ngorora location at Kabarsero, one and a half miles (2·4 km) north of Chepkesin, 0° 54′ N, 35° 51′ W (Fig. 1). A sedimentary sequence over 1,200 feet (360 m) in thickness conformably overlies a thick massive non-porphyritic phonolite flow. The whole succession dips at between 18° and

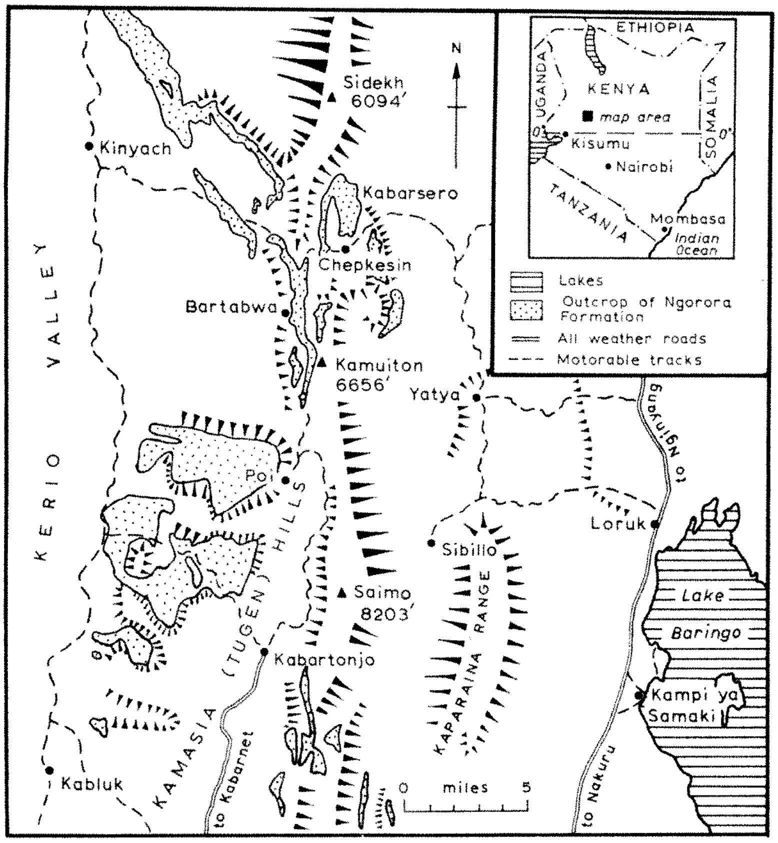

Fig. 1. Map showing the extent of the Ngorora Formation.

20° to the west. Five lithological subdivisions are recognized (*A* to *E*, Fig. 2).

E, diatomites, paper shales and coarse grit	350 feet +	105 m
D, rhythmic units with fossiliferous channel-conglomerates	220 feet +	66 m
C, porcellanous cream and green tuffaceous silts with pumiceous horizons	200 feet	60 m
B, rhythmic unit with fossiliferous channel-conglomerates and grits	140 feet	42 m
A, clays and coarse red tuffs	310 feet	92 m

A.—Variable bedded tuffs, tuffaceous clays and grit* lenses. The outstanding beds are massive tuffs with lava blocks which occur near the top and the base of the subdivision. These are stained red in patches but the red coloration is not as persistent in the type area as at other localities.

B.—Ferruginous pebbly grit and a prominent bed with tuff-boulders mark the base of this division. Above these beds, sedimentation is in the form of alternations of current-bedded grits, sands and clays with bedded pumice tuffs and tuffaceous silts. Because rhythmic sedimentation is typical of division *D* also, a standard bipartite unit is illustrated (Fig. 3) and described in more detail.

The lower part of this generalized unit shows a decrease upwards in the number of grit bands and in overall grain size. Vertebrate fossils are concentrated in the conglomerate and grit lenses and are often very abundant. Bones and teeth show a variable degree of rolling but are normally well preserved. (The principal fossil mammal horizons are indicated in Fig. 2, but fragments of fish occur in sandy beds throughout the sequence.) Well preserved chelonian remains occur more generally in the silts and clays of the middle of the unit. These are succeeded by glutinous clays containing kunkar and occasional rootlet casts. These clays are characteristic of palaeosols produced by the weathering of tuffaceous silts. Sedimentary dykes of silicified angular pumice tuff also occur marking infilling of open cracks on land surfaces. The dykes invariably terminate at one of the palaeosol horizons.

The upper part of the unit is composed of thin-bedded pumice tuffs, occasionally showing trough cross-bedding but typically very evenly bedded and fine-grained with a sharply defined base (see Fig. 3). These strata usually form cliffs. The fine grained sediments are frequently silicified to a brittle porcellanous rock with chert bands. Fragments of wood and fruits occur in the tuff above Loc. *2V/11*. The top of the bedded tuffs is often cut by channels containing the basal grit and conglomerate of the succeeding unit. This feature is well shown below Loc. *2V/1* where the fossil bearing deposits lie in channels which have cut through a massive accretionary lapilli-tuff producing pot-holes and water-worn blocks.

C.—This is essentially the upper part of the rhythmic unit of Loc. *2V/1*. It has been mapped separately to facilitate the study of thickness variations. It consists of cream and green well bedded tuffaceous silts exhibiting variable silicification and containing five coarser pumiceous horizons.

D.—Comprises three rhythmic units of which the lower two contain the fossil localities *2V/10* (the "Charnel House Grit"), *2V/11* and *2V/13*. The top of the sands and clays of Loc. *2V/11* is marked by a thin, 1·5 inch (40 mm), but laterally persistent pebble bed. This and the underlying sands were subjected to minor faulting and fissuring before the deposition of the overlying bedded tuffs.

E.—White diatomites and finely laminated diatomaceous and tuffaceous silts occur at the top of the sequence in the Chepkesin–Kabarsero area. Above this sequence is a prominent massive cross-bedded grit and conglomerate overlain by a thick tuffaceous silt; the grit and conglomerate make a prominent cliff above the road south of the old Chepkesin village.

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* "Grit" is used as a convenient term to denote characteristic coarser feldspathic/lithic sandstones.

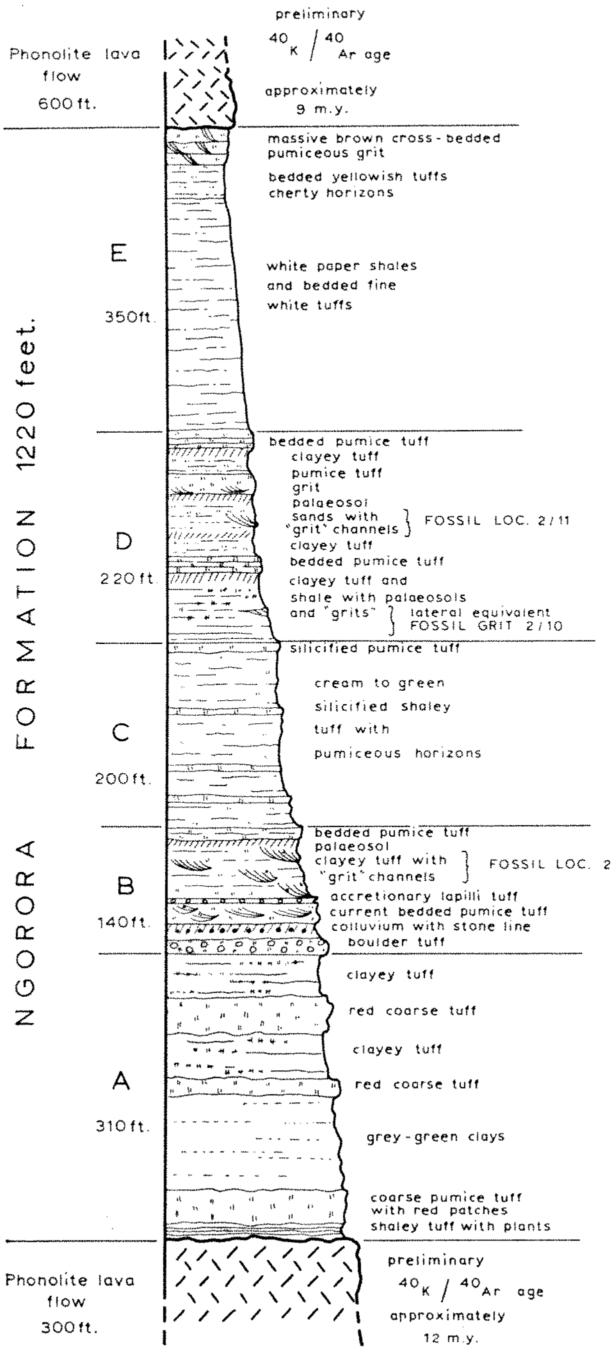
Phonolite lava flow 600 ft.
preliminary
40K / 40Ar age
approximately 9 m.y.
massive brown cross-bedded pumiceous grit
bedded yellowish tuffs cherty horizons
E
350 ft.
white paper shales and bedded fine white tuffs
NGORORA FORMATION 1220 feet.
bedded pumice tuff
clayey tuff
pumice tuff
grit
palaeosol
sands with "grit" channels
FOSSIL LOC. 2/11
D
220 ft.
clayey tuff
bedded pumice tuff
clayey tuff and shale with palaeosols and "grits"
lateral equivalent FOSSIL GRIT 2/10
silicified pumice tuff
C
200 ft.
cream to green silicified shaley tuff with pumiceous horizons
bedded pumice tuff
palaeosol
clayey tuff with "grit" channels
FOSSIL LOC. 2/1
B
140 ft.
accretionary lapilli tuff
current bedded pumice tuff
colluvium with stone line
boulder tuff
clayey tuff
red coarse tuff
clayey tuff
A
310 ft.
red coarse tuff
grey-green clays
coarse pumice tuff with red patches
shaley tuff with plants
Phonolite lava flow 300 ft.
preliminary
40K / 40Ar age
approximately 12 m.y.

Fig. 2. Lithological divisions of the Ngorora Formation in the type section at Kabarsero.

The sedimentary sequence is overlain by a massive phonolite, the youngest of the "plateau phonolite" group in the area.

Other Areas

Sections of the Ngorora Formation exposed on the crest and dip slope of the Sidekh hills, three miles to the west of Kabarsero, on the up-throw side of the Chepanda Fault (2,000 feet (600 m) throw) are only 100–150 feet (30–45 m) thick. Also a progressive thinning to 250 feet (75 m) is observed when the formation is traced south-south-westwards from Kabarsero toward the Saimo range (Fig. 1). There is everywhere a close correlation between the thickness of the formation and structural relief. Massive reddened tuffs characterize the basal horizons and a general increase of fine-grained well bedded lithologies occurs upwards.

When traced south and west from the type area, the Ngorora Formation is seen to have complex interdigitating relationships with the Poi Tuffaceous Sandstones and Kapkiamu Shales of Martyn[2], and lithologies identical to those of the Ngorora type-section comprise the southern outcrops of the Poi Tuffaceous Sandstones. It is more satisfactory to treat all these sediments, which mapping has shown to be a single lithosome, as one formation within which the details of the lateral variations remain to be resolved. It is therefore proposed that the Ngorora Formation should include the Poi and Kapkiamu units of Martyn.

Table 1

PISCES			*Clarias* sp. *Tilapia* sp.*
REPTILIA	Chelonia	Pelomedusidae	*Pelusios*
	Crocodilia		
AVES		Ciconiidae	
MAMMALIA	Primate	Hominoidea	hominid ?
		Cercopithecoidae	
	Carnivora		
	Proboscidea	Gomphotherid mastodont	
		Deinotheridae	*Deinotherium* sp. Sp. nov.
	Hyracoidea		
	Perissodactyla	Chalicotheriidae	
		Rhinocerotidae	*Chilotherium* sp. *Aceratherium* sp. ? *Brachypotherium* sp.
	Artiodactyla	Suidae	
		Hippopotamidae	
		Giraffoidea sp.	
		Giraffidae sp.	
		Boselaphini	? *Protragocerus* sp.
		Tragelaphini/ Boselaphini sp.	
		Cephalophini/ Boselaphini sp.	
		Caprinae/ Alcelaphini sp.	

* Identified from the Kapkiamu Shales (see text).

Environmental Interpretation

Preliminary examination of cross-bedded structures, imbrications and channel orientations suggests sediment transport from between north and east. This seems to preclude derivation from the Kamasia itself, but thickness variations in the sediments show that the range was obviously a positive tectonic element within the basin of deposition.

Division *A* of the type section indicates early pyroclastic activity and several of the massive red tuffs with blocks may be lahar deposits. Units *B* and *D* are interpreted as the sedimentological results of movements on the rift faults bounding the depositional basin. The postulated sequence of events governing a rhythmic unit is: (i) Fault movement within the depositional basin producing tilting of the floor, with consequent channel erosion and deposition of coarse epiclastic sediments. (ii) Slight persistent movements tending to negate the previous tilting and resulting in progressively lower energy environments of swamps and alluvial plains with deposition of silts and clays. Fully subaerial conditions are represented by palaeosols and occasional primary tuffs which are seen locally to have been incised by stream channels. (i) and (ii) together produced the lower (sand/clay) part of the generalized unit (Fig. 3). (iii) Further faulting caused rapid transgression of lake waters across the alluvial plains and deposition of the pumiceous pyroclastics (probably originating within the Rift) in stillwater conditions. The wood and fruit in these sediments, and also the pumice fragments, would have been able to float to some distance from the shore. No mammalian fossils were found in this upper part of the unit.

Divisions *C* and *E* represent more stable periods of lacustrine deposition, the laminated diatomaceous tuffs of *E*, in particular, indicating an episode of tranquil lake conditions which was eventually terminated by the deposition of the conglomerate and grit at the top of the sequence.

Mammalian Fauna

The fossils collected from the Ngorora Formation include fish, reptiles, bird and mammals. Those identified to date are shown in Table 1.

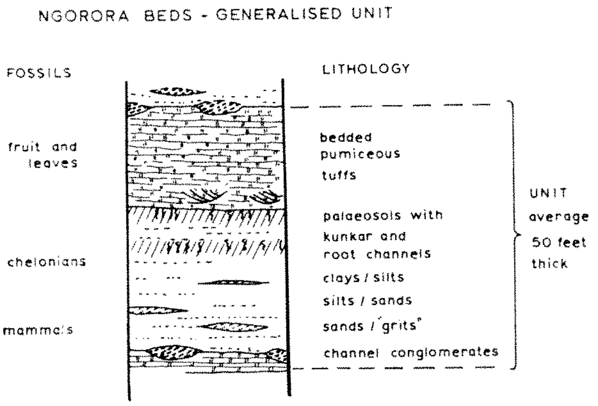

Fig. 3. Generalized unit typical of rhythmic sedimentation in divisions *B* and *D* of the Ngorora Formation.

Fossil plants also occur in the formation in the form of leaf and stem impressions, rootlet casts, fossil wood and occasional moulds of fruits.

The preliminary faunal list is based on specimens collected from more than twenty individual localities occurring within the 19 to 20 square miles of outcrop shown north of Kamuiton summit (6,656′) in Fig. 1. The majority of the localities fall, however, within an area of 2·5 square miles traversed by the type section at Kabarsero. Within the vertical sequence (Fig. 2), all but one of these fossil mammal localities occur in the channel grits and conglomerates of units *B* and *D*, and, as a result, the specimens are always in a dismembered, broken and sometimes rolled condition. The hominid tooth crown, in contrast, was from a manganese palaeosol horizon. The marabou stork skeleton was found partially articulated in a biotite bearing tuffaceous silt and seems to suggest rapid sedimentation in still water conditions well away from a channel.

Until larger collections are available and the existing specimens have been studied in detail it would be dangerous to attempt to establish an age for the mammalian fauna based on assessment of its stage of evolution, but some preliminary comments may be made on the nature of the fossils. We are indebted to Dr L. S. B. Leakey for the following note on the hominid tooth.

"The specimen consists of the crown of a left molar from which the roots have broken off (Fig. 4). The crown exhibits a well developed trigon with a rather large hypocone which is separated from the trigon by a relatively deep fissure. Both anterior and posterior contact facets are present. The former is rather larger than the latter, but its shape and size suggest contact with a molar rather than a premolar tooth, although this cannot be excluded. On the available evidence it may be assumed that the tooth is a second upper left molar. The crown is very low relative to the overall size, but this is not the result of wear on the cusps which show almost no attrition. On the lingual face a valley extends down to about half way point, while on the buccal face this valley is much less marked and ends in a very slight pit. There is a clear but small posterior fovea and a minute trace of an anterior one. Nowhere is there any trace of a cingulum. The tooth is fairly large, its measurements being length 12·35 mm; buccolingual width 14·00 mm. The posterior face is rather curved, while the anterior face is almost straight. Lingually, the enamel slopes slightly from the top of the cusps towards the neck of the tooth. A similar but not quite so marked feature is to be seen on the buccal aspect. At first sight this crown resembles the genus *Kenyapithecus*. Closer examination, however, shows characters which are suggestive of an evolutionary trend towards both *Homo* and *Australopithecus*. In fact, on morphological grounds, it would be possible to treat this specimen as a slightly aberrant tooth of either *Australopithecus africanus* or of *Homo habilis* except for the height of the crown which is distinctly lower relative to tooth size than is to be seen in teeth of either of these genera. It seems wisest at this stage to regard this specimen as representing a member of the Family Hominidae, having some affinities to *Kenyapithecus* (such as the very low crown) and some to both *Homo* and *Australopithecus*."

The other fossils will not be described in detail here. It should be noted, however, that rhinoceros material is abundant in most of the channel situations of unit *D*, and represents at least two and probably three genera. The absence of equid material from the collections made so far may prove significant as in the younger Baringo assemblages, aged seven million years or less, equids are represented in the form of *Stylohipparion*.

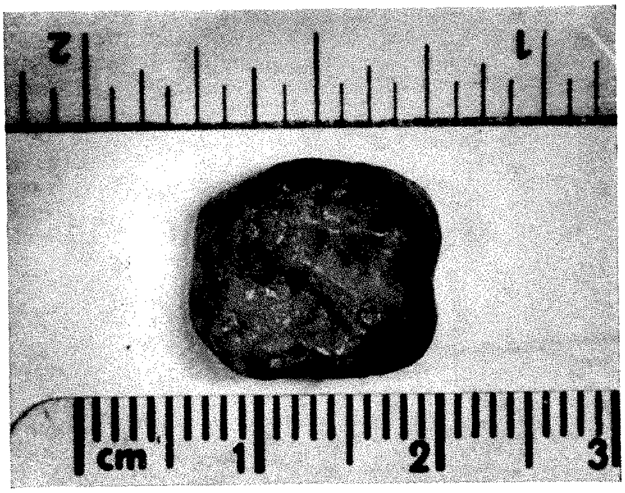

Fig. 4. Crown view of left upper molar of hominid from the Ngorora Formation.

The presence of horned bovids and of giraffoids in the artiodactyl assemblage suggests that at this time they were still in competition for similar niches in the environment. It is also worthy of note that these strata dated in excess of 9 m.y. contain teeth already having a recognizably hippopotamid morphology. These are at present the oldest dated "hippo" teeth, but the material is very sparse when considered as a percentage of the total assemblage in contrast to the dominance of hippo fossils in many Rift Valley localities of later date. This is probably of environmental rather than evolutionary significance.

Fish are represented by the individual bones and scales of dismembered skeletons at numerous horizons interspersed with and usually having an "overbank" relationship to the main Ngorora mammal bearing channels. As can be seen, however, from the faunal list, *Tilapia* has been identified from the Kapkiamu Shales. These fossils occur, frequently as complete skeletons, on the bedding planes of the shales. The individuals are small in size and limited in size distribution (80 to 100 mm long) which may be the result of adaptation in an alkaline lake basin of limited extent.

Chelonians are usually small and occur as fragments of skeleton and carapace which have broken up consequent upon mass movement in the tuffaceous clays. One large chelonian carapace (1·5 m long) was noted in a channel conglomerate of unit *D*.

We are indebted to the following who are undertaking detailed studies of the groups indicated: Dr A. Gentry (Bovidae and Giraffoidea), Dr J. Harris (Deinotheriidae), Mr A. Hill (Hyracoidea), Dr D. A. Hooijer (Rhinocerotidae), Dr L. S. B. Leakey (Primates), Mrs S. C. Coryndon Savage (Hippopotamidae), Mrs J. Van Couvering (Fish), Dr A. C. Walker (Ciconiidae) and Mr A. Wood (Chelonia).

Age and Palaeomagnetism

Potassium-argon ages are being established by Dr J. A. Miller in the Geochronological Laboratory, Department of Geodesy and Geophysics, University of Cambridge. Only preliminary dates are available at present (Fig. 2). It is not proposed to comment further on these ages beyond noting considerable variation in repeat runs and large probable errors, because of the presence of calcium carbonate and zeolites in the phonolite lavas. Further samples of the bracketing potash-rich phonolite lavas, together with sanidine feldspars from angular pumice fragments in units *B* and *D*, should allow accurate dates to be established for the fossiliferous horizons.

Although firm dates are still awaited from Ngorora itself, the formation and the overlying phonolite and basalt lavas are unconformably overlain by flows of Kabarnet Trachyte. Potassium-argon dates for these trachytes are in good agreement with each other and have small probable errors. They suggest an isotopic age of 7·0± m.y. as a firm upper age limit for the Ngorora Formation plus superincumbent lavas. When mapping evidence of unconformity is taken into account, an age in excess of 9·0± m.y. seems confirmed for the fossiliferous sediments. The age of 12·0± m.y. or younger for the underlying phonolite also receives support by extrapolation from the ages obtained for the plateau phonolites of the Uasin Gishu escarpment[3].

A model 70 portable fluxgate magnetometer was used to establish polarities for lava flows overlying and underlying the Ngorora Formation. The field results show good agreement in the polarities obtained for up to six readings on each flow unit, but these are being checked against "cleaned" laboratory readings. The lavas overlying the Ngorora Formation, including the Kabarnet Trachytes, yield normal polarities in the field although to the north of Kamuiton Hill (Fig. 1) a thin reversed flow may occur between the normal flows and the sediments. The underlying phonolites yield reversed field polarities.

Significance of the Ngorora Formation

The Ngorora Formation is interpreted as the product of intermittent fluvio-lacustrine deposition, involving both the eroded debris of earlier volcanism and the redistribution of coeval pyroclastic material on an intermittently subsiding rift floor.

The fossils fall in the early Pliocene (or possibly late Miocene) time span for which nothing is known of mammalian evolution in Africa south of the Sahara. The fauna helps to bridge the gap between the Fort Ternan assemblage (14·0± m.y.), which also includes horned bovids and giraffoids, and the earliest mammalian assemblages with australopithecines from the Omo and East Rudolf areas of the northern Gregory Rift (2·0± to 4·0 m.y.). The search for further hominid material from this time span is of critical importance.

We thank Dr L. S. B. Leakey and Professor E. Aguirre for kindly undertaking palaeontological work in the Ngorora area in July 1969. Chief Harun Loyetta provided valuable local assistance and Andrew Hill, Paul Buckland and Edward Spooner helped with the field work. The field work was supported by grants from the Kenya Government, the Natural Environment Research Council, the Wenner-Gren Foundation for Anthropological Research and the Boise Fund of Oxford University.

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Geology and Fauna of a New Pliocene Locality in North-western Kenya

by
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Lothagam Hill reveals a succession of fossil bearing sediments that extend knowledge of the East African Pliocene back from the base of the Omo succession, to about 5·5 million years

In 1967, an expedition from the Museum of Comparative Zoology, Harvard University, worked a new Pliocene locality at Lothagam Hill in south-eastern Turkana District, Kenya. Fossils had been noticed there by Dr Lawrence H. Robbins in the course of archaeological excavations carried out in 1965. A preliminary geological map of the locality was made by W. D. S. and B. P. in 1967, and more detailed work was carried out by A. K. B. in 1968. Studies of the faunas are in progress and a detailed report by A. K. B. on the geology is in preparation.

Lothagam Hill is an isolated, westward-tilted fault block situated in the plain adjacent to Lake Rudolf between the Kerio and Lomunyenkupurat Rivers (Fig. 1).

It is situated at approximately 2° 53′ N latitude and 36° 04′ E longitude, covers about 10 square miles and has a maximum relief of 200 m above the plain, which in turn is about 370 m above sea level. The block is composed of Miocene and Pliocene volcanics and sediments with major normal faults running north–south along the eastern edge. The topography is controlled by two relatively resistant volcanic units. These form roughly parallel north–south ridges, with sediments between and to the west (Fig. 2). Overlying these older rocks unconformably are horizontal, subrecent deposits from a former high stage of Lake Rudolf. A preliminary account of the archaeology of these late deposits has been given by Robbins[1], who assigns them to Fuchs's 220 foot level, and they have been dated by carbon-14 at about 5,000 yr BP (personal communication from L. H. Robbins).

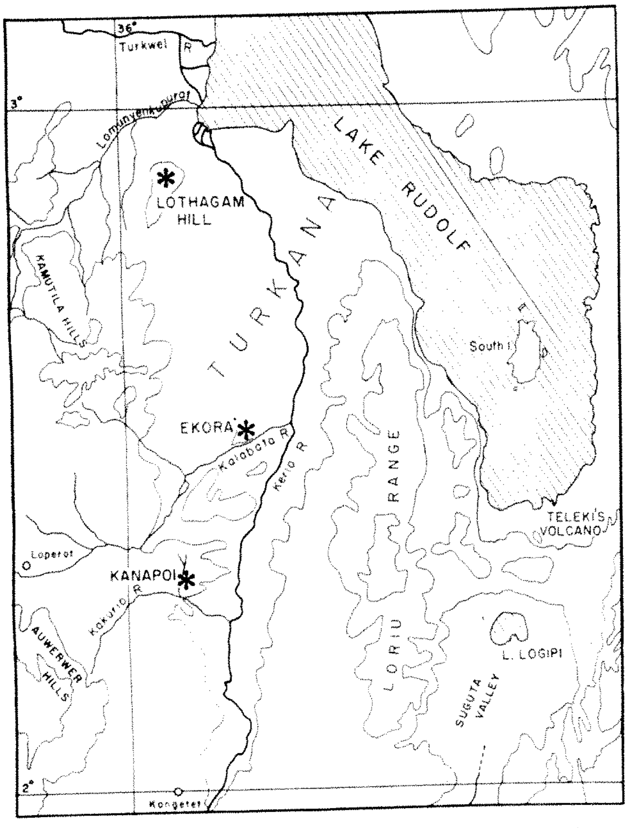

Fig. 1. Map showing position of the Lothagam, Kanapoi and Ekora localities.

Stratigraphy

A generalized stratigraphic column is given in Fig. 3. The total thickness of the Lothagam section exceeds 950 m, and the sediments make up about 720 m of this. The lowest exposures consist of two series of lava flows with some interbedded water-laid deposits. These series are separated by a major disconformity and may be referred to as the Miocene and Pliocene volcanics on the basis of whole rock K–Ar determinations: 16·8 ± 0·5 m.y. on a Miocene flow (Geochron R-0906), and 8·31 ± 0·25 m.y. on a Pliocene flow (Berkeley JA-2294). Conglomerates and water-laid tuffs become increasingly common toward the top of the Pliocene volcanic series, and the boundary between this and the overlying fossiliferous sediments is set at a disconformity on the uppermost basalt flow. The only fossils encountered were pieces of silicified wood in the Miocene volcanics and a few shells of *Etheria elliptica* in the Pliocene.

The overlying sedimentary deposits form three distinct lithological units. A basalt sill some 30 m thick (the first to be recorded for the East African late Caenozoic) has been intruded between the first and second of these, and postdates the sediments. The sill has yielded a whole rock K–Ar date of 3·71 ± 0·23 m.y. (Berkeley KA-2262), thus placing an upper limit on the age of the sedimentary sequence as a whole; it shows reversed polarity (personal communication from P. K. S. Raja), and may have been intruded early in the closing third of the Gilbert Reversed Epoch[2]. The sedimentary sequence and the basaltic sill may be designated as the Lothagam Group, and the three sedimentary units, from bottom to top, as Lothagam-1, 2 and 3.

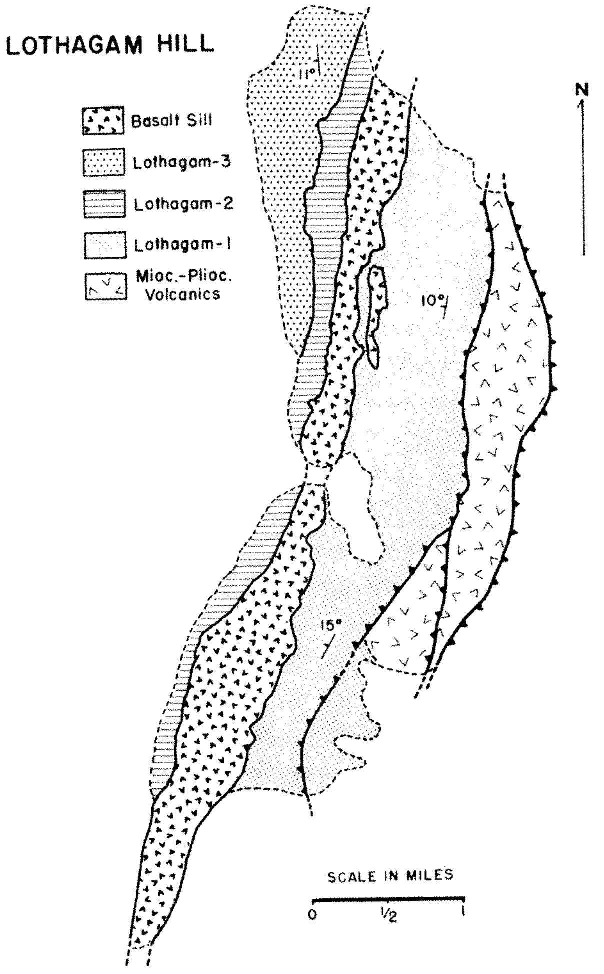

Fig. 2. Simplified geological map of Lothagam Hill; subrecent sediments are not shown and minor faults have been omitted.

Lothagam-1. This unit, the lower fossiliferous beds of Maglio[3], has produced the richest and best preserved fauna. It is 480 m thick in the north and 370 m thick in the south, and has the general characteristics of a clastic wedge deposited on the edge of a rapidly subsiding basin. This could well have been the ancestral Lake Rudolf Basin, opening out to the north. There is no geological or faunal evidence for any major time breaks within the formation; deposition was apparently continuous, although at varying rates. The deposits are clearly fluvio-deltaic in origin, and indicate the presence of at least one major river system as a transporting and depositing agent. Sediment types range from coarse, poorly sorted conglomerates and sandstones to fine silts and laminated tuffaceous shales. The sedimentary structures and bedding associations indicate deposition in channel, floodplain and backwater environments. Water flow throughout the year rather than seasonally is suggested by the molluscan fauna (especially banks of large *Etheria elliptica*) and the absence of evaporite deposits. Lithological differences permit three subdivisions of Lothagam-1, which have been designated in the section as Members *A*, *B* and *C*, from bottom to top. The lowest, *A*, consists of coarse, non-fossiliferous conglomerates and sandstones, the middle of alternating sandstones and silts, and the upper of well sorted channel sandstones with some silt lenses. Member *B* contains the richest fossil fauna in the northern part of the exposures, while Member *C* is most prolific in the south. The uppermost beds of *C* are baked as a result of the intrusion of the sill.

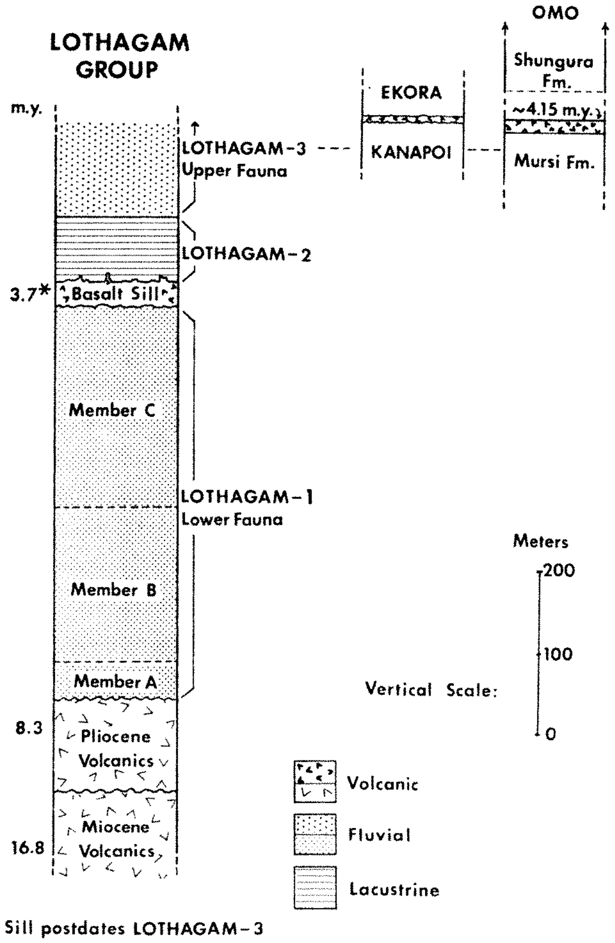

Fig. 3. Columnar section of the Lothagam succession and its relations to Kanapoi, Ekora and the base of the fossiliferous Omo succession. For broader correlations, see Maglio[3].

Lothagam-2. This unit is an 80 m thick sequence of fine grained silts, clays, and occasional water-laid tuffs indicating a change to a lacustrine environment of deposition. Fossil vertebrates (hippopotamus, crocodile, turtle, fish) are rare, but beds of gastropods up to a metre thick are fairly common; identified gastropods are: *Bellamya unicolor*, *Caelatura* ? sp. B and *Cleopatra bulimoides*. Gypsum and salt in the deposits are evidence for at least partly saline conditions. The lower beds of the unit are contorted and baked, again as a result of the intrusion of the sill.

Lothagam-3. This unit, the upper fossiliferous beds of Maglio[3], lies conformably on Lothagam-2 and demonstrates a return to a fluvial environment of deposition. The top is not exposed because it is concealed beneath subrecent to recent alluvium, but the unit exceeds 90 m in thickness. The sediments are similar to Member *C* of Lothagam-1, consisting of coarse sandstones and floodplain-type silts and clays. Vertebrate fossils occur, although because of low relief and deeply weathered exposures they are much fewer in number than those found in Lothagam-1. There are no apparent faunal or stratigraphic breaks within the unit.

Fauna

The fauna of Lothagam-1, so far as identified, is as follows: Cercopithecidae indet., *Australopithecus* sp. cf. *A. africanus*, Anomaluridae nov., Hyaenidae indet., cf. *Civettictis*, Felinae indet. (a large form), Machairodontinae indet., Orycteropodidae gen. et sp. nov., *Deinotherium* sp., Gomphotheriidae indet., *Stegotetrabeledon orbus*[4], *Primelephas gomphotheroides*[4], *Stylohipparion* sp., *Brachypotherium* sp. nov., *Nyanzachoerus* spp. nov. B and C, *N.* sp. cf. *N.* sp. nov. A, *N.* sp., *Hippopotamus* (*Hexaprotodon*) spp. nov. A and B, *Giraffa* sp., *Tragelaphus* sp. cf. *T. nakuae*, *Gazella* sp., *Redunca* sp. aff. *R. ancystrocera*, other Bovidae, *Crocodylus* sp., *Euthecodon* sp., Squamata indet., Pythoninae indet., *Podocnemis* spp. nov. A and B, *Geochelone* sp., Trionychidae indet., fish, *Bellamya* ? sp., *Ampullaria wernei*, *Lanistes carinatus*, *Caelatura* ? sp. A, *Melanoides* sp., *Etheria elliptica*. The mammalian assemblage is clearly earlier than that from Kanapoi. Only one species, *Nyanzachoerus* sp. nov. B, is at present definitely known to be common to the two (personal communication from H. B. S. Cooke and R. F. Ewer). The elephants of Lothagam-1 are more primitive than those characteristic of Kanapoi; granting that *Primelephas* is ancestral to later forms[3,4], an estimate of 1·0 to 1·5 m.y. for the time interval between deposition of the two formations is not unreasonable. Lothagam-1 provides a very useful standard against which to assess the relative ages of other faunas. It seems to be slightly older than Kolinga and lower Kaiso, equivalent, at least in part, to Kaperyon (which seems to be related to trachytes radioactively dated around 5 m.y.; personal communication from W. W. Bishop) and somewhat younger than Sahabi[3]. 5·0 to 5·5 m.y. would seem to be a plausible estimate for the age of Lothagam-1.

The few fossils found in Lothagam-2 are useless for purposes of correlation; the differences between the molluscs of this formation and of Lothagam-1 are the result of environmental factors—lacustrine vs. fluvio-deltaic depositional environments.

The scanty fauna of Lothagam-3 consists of *Simopithecus* sp., *Loxodonta adaurora*[4], *Stylohipparion* sp., Hippopotamidae indet. (a fragment), *Nyanzachoerus* sp. nov. B, *Notochoerus* sp. cf. *N. euilus*, *Tragelaphus* sp., other Bovidae, *Crocodylus* sp., *Euthecodon* sp., *Podocnemis* sp. nov. A, Trionychidae indet., fish, *Etheria elliptica*. Apart from *Simopithecus* (represented by a single tooth), all these forms occur in the Kanapoi and correlation with that fauna seems reasonably certain.

Age of the Kanapoi Fauna

The age of the fauna from the Kanapoi Formation now requires re-examination. When first reported, all that could be said of it was that it was pre-Olduvai and might be of early Pleistocene, "Villafranchian", age[5]. This estimate seemed to be nearly in accord, within the limits of the error, with a whole rock K–Ar date of 2·9 ± 0·3 m.y. (Geochron R-0554) for the overlying basalt, which exhibits reversed polarity. A subsequent date of 2·5 ± 0·2 m.y. (Geochron R-0554) seemed to accord perfectly with the palaeomagnetic evidence[6]. Recently, a third date of 2·71 ± 0·26 m.y. (Berkeley KA-2261) has been obtained. Since publication of the first two dates the recognized number of magnetic reversals has increased considerably[2], and it is now uncertain, in view of the spread of the dates, as to whether the Kanapoi basalt falls early in the Matuyama Reversed Epoch or in either the Kaena or the

Mammoth Events of the Gauss Normal Epoch. Recent findings have, however, made it clear that none of the dates accords with the evidence of the fauna. A revised, but far from final, faunal list of the Kanapoi is as follows: *Parapapio jonesi*[7], cf. *Australopithecus*[6], *Lepus* sp., *Hystrix* sp., *Tatera* sp., *Enhydriodon* sp. nov., *Hyaena* sp., Machairodontinae indet., *Deinotherium bozasi*, *Anancus* sp., *Stegotetrabeledon* sp. ? (fragments)[3], *Loxodonta adaurora*, *Elephas ekorensis*[4], *Stylohipparion* sp., *Ceratotherium* sp. nov., *Nyanzachoerus* spp. nov. A and B, *Notochoerus* sp. cf. *N. capensis*, *N.* sp. cf. *N. euilus*, *Hippopotamus* (*Hexaprotodon*) sp. nov. C, *Okapia* sp., *Giraffa* sp., *Tragelaphus* sp., *Reduncini* ? sp., other Bovidae, *Crocodylus* sp., *Euthecodon* sp., *Podocnemis* sp. nov. A, *Geochelone* sp., Trionychidae indet., fish, molluscs. If the ages obtained for the basalt accurately reflected the age of the sediments beneath it, this fauna should correlate approximately with that associated with Tuff *D* (ref. 8) in the Shungura Formation[9] of the Omo sequence. It does not, being notably more primitive. Like Lothagam-1, Kanapoi now provides a standard of comparison for other faunas. It may be correlated, in whole or in part, with Kanam, Chemeron, early Laetolil and Mursi (Yellow Sands)[3,4,10]. The last of these is capped by a basalt radioactively dated at 4·05 ± 0·2 m.y.[8] and 4·25 m.y.[9]; this dating falls into sequence with 3·75 ± 0·2 m.y. obtained for Tuff *B* (ref. 8) near the base of the Shungura Formation, and 3·95 ± 0·11 m.y.[11] for the unfossiliferous Nkalabong Formation[9]. An age between 4·0 and 4·5 m.y. for the Kanapoi Formation is a reasonable estimate on available evidence. The discrepancy between the radioactive dates for the Kanapoi basalt and the date indicated for the fauna is a question to be settled by future work. The basalt may be a sill. Where exposed its upper surface is weathered, but it is possible that detailed investigation around the Ekora anticline might decide the matter. If a sill, it is a rather extensive one.

The Ekora Sediments

The 1967 expedition recognized a yet higher sedimentary unit that overlies the Kanapoi basalt. Reconnaissance in 1966 had traced the Kanapoi sediments and lava northward to Ekora, just south of the junction of the Kerio and Kalabata Rivers, where they are involved in a low, east–west trending anticline; patches of sediment overlying the basalt were seen on the north limb of the anticline[6].* This structure plunges westward at a low angle and the basalt dips under immediately west of the Kalabata River. The area to the west of this point is largely covered by superficial deposits and a scanty vegetation cover, but patches of underlying sediments are here and there exposed. Fortunately, in the immediate vicinity of the basalt, a moderately resistant bed of orange-brown grit occurs; this could be traced around the nose of the basalt and observed to be concordant with it in dip and structure, thus showing that the sediments participate in the anticline, overlie the basalt and are hence younger than Kanapoi. No continuous section of them is available, the exposures are poor and the top is not visible. They appear to be predominantly fine-grained with some beds of sandstone and grit; the finer sediments contain fragmentary remains of fish and other aquatic vertebrates and a few terrestrial forms have been recovered from the coarser sediments. The fauna consists of *Anancus* sp., *Loxodonta adaurora*, *Elephas ekorensis*, *Ceratotherium* sp. nov., *Nyanzachoerus* sp. cf. *N.* sp. nov. B, *Euthecodon* sp., *Podocnemis* sp. nov. A, *Geochelone* sp., fish and molluscs.† This assemblage is consistent with the stratigraphic data and represents a continuation from the Kanapoi fauna. (This demonstrates that the Kanapoi basalt was not extruded some 2·7 m.y. ago over an old surface of Kanapoi sediments.) The elephants suggest that the Ekora may be older than the Shungura. *Elephas ekorensis* is ancestral to *E. recki*, and an early stage of that species is present in the lower part of the Shungura at Tuff *B* (ref. 3). Even with a generous extension of Pleistocene time—to the base of Etouaires in the European Villafranchian succession at 3·3 m.y.[12]—Lothagam, Kanapoi and Ekora are clearly of Pliocene age.

A final point concerning this sequence of faunas is that the fishes and molluscs occurring in them have, like those in the Omo sequence[9], chiefly nilotic affinity. This indicates a drainage system then open to the north. These formations have been affected by diastrophic movements that, among other things, resulted in the general westward dip of the rocks in the region. These tectonic events, which gave the Lake Rudolf basin its present configuration, evidently ended in Pleistocene, possibly mid-Pleistocene, time.

The East African Pliocene

In the past five years there has been a remarkable increase in knowledge of the continental late Caenozoic in East Africa. Well established chronology previously ended at the base of the Olduvai sequence. Isolated localities—Kanam, Kaiso, Laetolil, Kanapoi (when first discovered)—were recognized as or suspected of being pre-Olduvan in age, but there was no standard against which they could be compared. The recent understanding of the Omo succession[8,9], which overlaps Olduvai in time and extends back beyond 4 m.y., has helped enormously in this connexion. Ekora, Kanapoi and Lothagam, which in turn overlap Omo, carry the chronology back to around 5·5 m.y. or more. Recent work in the Baringo basin has made known fossil bearing sediments that correlate at the top with Kanapoi and Mursi and reach back, although separated by gaps, to beyond 9·0 m.y.[13]. The East African Pliocene, hitherto unknown or essentially unrecognized, is now a reality.

The field work was supported by the US National Science Foundation; a grant from the Milton Fund of Harvard University has helped to defray the costs of radioactive dating. We thank our companions in the field, A. D. Lewis, R. C. Wood, V. J. Maglio, J. H. Wahlert, C. T. Williams, A. E. Greer, D. M. Ngumi, S. Rueben and M. Nathale; the Ministry of Natural Resources, Kenya; Dr and Mrs L. S. B. Leakey and the staff of the National Museum Centre for Prehistory and Palaeontology; Dr J. Walsh and the staff of the Mines and Geological Department, Kenya; Dr and Mrs R. J. Drysdale-Anderson and the staff of the African Inland Mission, Lokori; and Mr Richard Leakey of the National Museum of Kenya. For comment, information and determinations we thank W. W. Bishop, H. B. S. Cooke and R. F. Ewer, S. Coryndon Savage, A. W. Gentry, D. A. Hooijer, F. C. Howell, B. Kurtén and E. Anderson, V. J. Maglio, P. K. S. Raja, K. S. Thomson, B. Verdcourt, J. Walsh and R. C. Wood. The potassium–argon dating was carried out by H. W. Krueger and N. J. Gilbert.

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* Sediments encountered to the south of Kachau Cone were then regarded as an extension of the Kanapoi. This now seems very doubtful. These isolated, horizontally lying, predominantly coarse deposits do not resemble anything found elsewhere in the area, and the few fossils collected from them—fragments of hippopotamus, giraffe and bovid—are unrevealing as to precise age. They are probably Pleistocene.

† *Simopithecus* sp.[4] was mistakenly listed previously; the specimen in question was found in Lothagam-3.

Bradford's Law and Library Acquisitions

by

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Bradford's law holds for periodical circulation as well as literature dispersion.

In a recent article[1], B. C. Brookes pointed out that Bradford's law of dispersion "seems to offer the only means discernible at present of reducing the present quantitative untidiness of scientific documentation, information systems and library services to a more orderly state of affairs capable of being rationally and economically planned and organized". This opinion is shared by others who have applied the Bradford and related distributions to various problems of library management; see, for instance, the survey by M. K. Buckland and A. Hindle[2]. Much of this untidiness is related to the lack of methods for determining the minimum size or lower limits of a useful collection of information sources. In this communication, we report on a method using Bradford's law to establish such lower limits for the purpose of maintaining a dynamic library collection. The upper limits, being essentially a function of budget, reach their own level by natural processes. We shall limit our discussion to the acquisition of periodicals in a medical library, although the method should be equally applicable to most scientific libraries.

Bradford's law of dispersion[3] states that, if the journals containing articles on a given subject are arranged in descending order of the number of articles they carried on the subject, then successive zones of periodicals containing the same number of articles on the subject form the simple geometric series $1 : n^2 : n^3 : \ldots$ Bradford called the first such zone the nucleus of periodicals particularly devoted to the given subject. Clearly, the nucleus is arbitrarily defined because it depends on the number of articles chosen to effect the division of periodicals into zones. Hence, Bradford's law, although an interesting phenomenon, was of little practical use. It was shown, however, that there is a minimal nucleus in the sense of Bradford[4,5]. That is, a core of journals can be separated from the top of an ordered list of periodicals dealing with a given subject which represents the most significant information sources for that field. This minimal nucleus, moreover, does not necessarily consist of only the most productive periodical, but may contain any number of journals depending on the extent of dispersion of the subject's literature among the various periodicals. Thus it is possible to set lower bounds on the number of journals which must be contained in a collection devoted to a given subject.

Nucleus of Journals

Brookes has also pointed out[1] that Bradford's law "can be expected to arise when selection is made of items, characterized by some common element, which are all equally open to selection for an equal period and subject to the 'success-breeds-success' mechanism". Hence, Bradford's law should apply to the use of periodicals in a library as well as to the dispersion of articles among journals. These are both acquisitions processes; namely, processes of obtaining relevant items by means of selection.

Table 1 shows the distribution of journal use in the Allen Memorial Medical Library, Cleveland, Ohio, during its peak month of March 1968. Use is here defined as circulation. Clearly, Bradford's law holds for this distribution. Table 2 shows the minimal nucleus and its successive zones of periodicals. These have been derived from consideration of the data about the least used titles, as was described in an earlier paper[4]. In general, the number of items effecting the finest Bradford subdivision must exceed one half the number of items of frequency one in the distribution. In this case, nine partitions would yield zones of periodicals each of which circulated about ninety-seven times, a number approximately equal to one half the number of periodicals which circulated once. Hence, eight zones constituted the finest subdivision of these data for which the Bradford hypothesis was valid. The minimal nucleus of journals circulated during March 1968 consisted of eleven titles that were borrowed 113 times, followed by sixteen periodicals circulating 108 times, twenty-one journals 107 times and so on. Consequently, successive zones of periodicals circulating about the same number of times formed the approximate geometric series $1 : (1{\cdot}4) : (1{\cdot}4)^2 : \ldots . : (1{\cdot}4)^7$. It is interesting to note that half the circulation for this month was accounted for by only seventy-six journals.

Table 1. JOURNAL CIRCULATION FOR MARCH 1968

No. of journals	Circulation per journal	No. of journals	Circulation per journal
1	18	12	6
1	12	15	5
2	11	28	4
1	10	36	3
3	9	75	2
7	8	186	1
4	7		

Table 2. MINIMAL NUCLEUS OF JOURNALS CIRCULATING IN MARCH 1968

Zone	Times circulated	No. of journals	Bradford constant (n)
1	113	11	—
2	108	16	1·5
3	107	21	1·3
4	110	28	1·3
5	110	38	1·4
6	110	55	1·4
7	109	93	1·7
8	109	109	1·2
	876	371	Average 1·4

Acquisitions Policy

Assuming that journal circulation in this library obeys Bradford's law, as seems to be the case, the following acquisitions policy can be formulated. In establishing such a policy it is important to base the selection of journals on expected future demand. Hence:

(1) First establish the minimal nucleus and its successive zones of periodicals circulating at various successive time intervals. The length of these intervals will vary from

library to library with the density of use. Some libraries may compute the nucleus each month, others every three months and so forth. This is necessary because the nucleus may vary with time as a result of a change in users or user interests.

(2) On the basis of these observations, establish the rate of change of circulation for each periodical circulating in the library.

(3) On the basis of these rates of change, establish by any appropriate means of extrapolation the expected circulation at some appropriate future date, say a year in advance.

(4) The expected minimal nucleus should then belong to the minimal collection in the library's inventory at that point in time.

As a safety factor it may be good policy for the library not to delete a given journal from its collection until its predicted decline in circulation is verified by fact.

Nucleus of Users

If the budget allows, it may be in the library's interest to include those periodicals which are of greatest interest to its best customers, provided they do not already appear in the minimal nucleus of circulating journals. Table 3 shows the distribution of users of journals in the Allen Library for the peak month of March 1968, and Table 4 shows the minimal nucleus and its successive zones of users during this time period. Use is again defined as circulation. The user distribution for March 1968 obeys Bradford's law whence the minimal nucleus of users consisted of thirteen individuals who borrowed journals 118 times, followed by eighteen individuals using periodicals 108 times, twenty-four individuals with 107 and so on. Thus successive zones of users borrowing about the same number of journals formed the geometric series $1:(1{\cdot}4):(1{\cdot}4)^2:\ldots:(1{\cdot}4)^7$. We note that half of the circulation was accounted for by only eighty-six users. We can thus

(1) Establish the minimal nucleus of users of the library.

(2) Determine the areas of interest of the members of this nucleus, in terms of *Index Medicus* subject headings. For medical researchers this can be done by selecting the headings from *Index Medicus* under which their most recent publications have been indexed. For clinicians, residents and interns this may be done by interest profiles.

(3) Establish the minimal nucleus for each subject heading represented by the user nucleus. This is done by applying Bradford's law to the distribution of articles among journals under each of the subject headings.

(4) The totality of such minimal nuclei should then be added to the minimal nucleus of circulating journals to form the library's minimal collection.

Table 3. DISTRIBUTION OF USERS FOR MARCH 1968

No. of users	Circulation per user	No. of users	Circulation per user
2	12	18	5
2	10	29	4
2	9	44	3
9	8	68	2
3	7	211	1
6	6		

Table 4. MINIMAL NUCLEUS OF USERS FOR MARCH 1968

Zone	Circulation	No. of users	Bradford constant (*n*)
1	118	13	—
2	108	18	1·4
3	107	24	1·3
4	109	31	1·3
5	109	40	1·3
6	108	54	1·4
7	108	105	1·9
8	109	109	1·1
	876	394	Average 1·4

Transplantation-Immunology Literature

That the distribution of articles among journals as indexed in the *Index Medicus* satisfies Bradford's law is shown in Tables 5 and 6, which give the distribution of articles and minimal nucleus of journals for the subject heading "Transplantation-Immunology" in *Index Medicus* for the years 1965–1968.

Table 5. DISTRIBUTION OF ARTICLES UNDER HEADING TRANSPLANTATION-IMMUNOLOGY

Journals	Articles per journal	Journals	Articles per journal
1	124	1	13
1	71	2	12
1	66	1	10
1	54	6	9
1	40	4	8
1	28	6	7
1	25	1	6
1	24	9	5
1	22	19	4
1	19	20	3
1	17	34	2
3	15	155	1

Table 6. MINIMAL NUCLEUS FOR TRANSPLANTATION-IMMUNOLOGY 1965–1968

Zone	Articles	Journals	Bradford constant (*n*)
1	124	1	—
2	137	2	2·0
3	122	3	1·5
4	122	6	2·0
5	122	11	1·8
6	129	20	1·8
7	126	35	1·8
8	118	74	2·1
9	120	120	1·8
	1,120	272	Average 1·8

The minimal nucleus for transplantation-immunology, not surprisingly, consisted of the single periodical *Transplantation*, the second zone contained the journals *Nature* and *Surgical Forum*, the third *Annals of the New York Academy*, *Proceedings of the Society for Experimental Biology and Medicine*, *Journal of Immunology* and so on.

Because it has been shown that Bradford's law applies to a portion of a literature as well as its totality[4], any part of the transplantation-immunology literature could have been chosen for this experiment. In practice, it would be best to take the most recent publications in establishing the minimal nucleus for a given field.

The expected minimal nucleus of users as well as the expected minimal nuclei of the subject areas of their interest at some appropriate future date can be established by extrapolation, and in this way the library can be in a position to anticipate future demand. In this case it may be a good policy to include new titles in the collection on the basis of these predictions.

In summary, it has been shown that the distributions of both circulating periodicals and their users in a medical library seem to obey Bradford's law. Hence, a smallest core of journals which must belong to the library's collection can be defined. This core should consist of the minimal nucleus of periodicals circulating in the library plus the minimal nuclei of journals devoted to the subjects of most interest to the library's nucleus of users. As the budget allows, successive zones of periodicals corresponding to circulation and user interest can be added. As a result, the library collection can be maintained in an orderly and viable state, thus providing its patrons with the most potentially usable materials for the funds at its disposal.

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LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Diamonds and the Interstellar Extinction Curve

IT has been suggested[1] that the interstellar extinction might be explained by assuming that the interstellar dust is composed of an appropriate size distribution of diamond particles. The imaginary part of the index of refraction of diamond increases sharply from near zero to about 1·0 at $\lambda^{-1} = 5{\cdot}0\ \mu\text{m}^{-1}$, giving an albedo near unity in the visible and strong extinction in the ultraviolet, as required by recent observations.

Wickramasinghe[2] has recently published a theoretical extinction curve for diamond particles, using a distribution of particle radii

$$n(a) \propto a^{-3} \qquad (1)$$

The curve passes within the error bars of the observations of interstellar extinction from $\lambda^{-1} = 1{\cdot}0$ to $8{\cdot}0\ \mu\text{m}^{-1}$ but gives too little extinction in the infrared and also results in a ratio of total to selective extinction, $R \equiv A_V/E_{B-V}$, of 10·8. Wickramasinghe therefore concludes that the hypothesis of dust grains made of diamonds is in conflict with the observations. The purpose of my paper is to show that a simple size distribution of diamonds may be found which accounts for the interstellar extinction.

I have made a series of exact Mie calculations with various simple size distributions and have found that the distribution

$$n(a) \propto a^{-3{\cdot}8} \qquad (2)$$

fits the observations throughout the infrared, visible and near ultraviolet regions fairly well. The ratio R for this distribution is 4·3, in agreement with the accepted range of values between 3 and 6. The number of very small particles is very large in such a distribution, however. Kimura[3] has shown that small dust grains may be expected to be destroyed by cosmic rays of energy 10–100 MeV if the grains are not able to thermalize the energy of the collision. I have therefore modified the size distribution to have the form

$$n(a) \propto a^{-3{\cdot}8}(1 - e^{-a/a_c}) \qquad (3)$$

A value of $a_c = 0{\cdot}03\ \mu\text{m}$ reduces n to 50 per cent of its value in distribution (2) at $a = 0{\cdot}02\ \mu\text{m}$ and to 10 per cent at $a = 0{\cdot}003\ \mu\text{m}$, and gives the best fit with the observations (see Fig. 1). For this distribution $R = 4{\cdot}9$.

The calculations were made by numerically integrating the extinction cross-section over the size distribution between particle radii 0·001 and 10 μm. The size of the increment in radius was made smaller at those wavelengths and radii where the cross-section shows rapid variations with the parameter $x = 2\pi a/\lambda$. The smallest increment used was 0·001 μm. The optical constants used were those of Phillipp and Taft[4].

In Fig. 1 is plotted the extinction in magnitudes, normalized to $V = 0$, $B - V = 1$, of the distribution (3) of diamond particles with $a_c = 0{\cdot}03\ \mu\text{m}$. Also plotted are the observations, similarly normalized, reported by Boggess and Borgman[5], those of Stecher[6] in the ultraviolet on five pairs of stars, those of Stecher[7] on one pair of stars, and those of Bless and Savage (private communication) made from the Orbiting Astrophysical Observatory on ten pairs of stars. The curve is nowhere more than one and a half error bars away from the observed points.

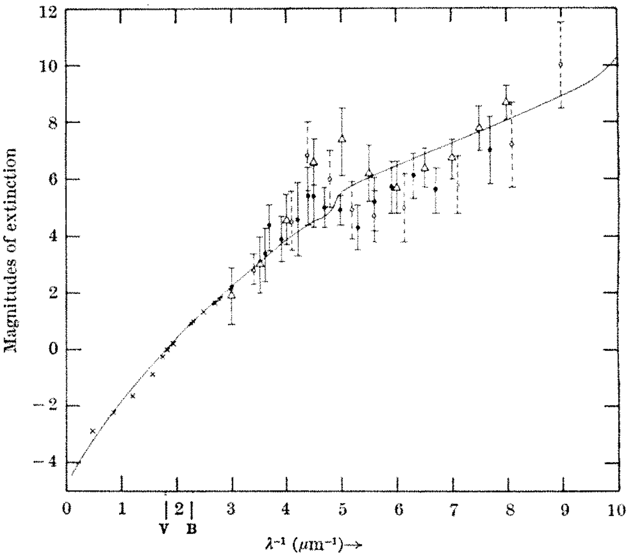

Fig. 1. Normalized extinction curve for a size distribution of diamond particles defined by $n(a) \propto a^{-3{\cdot}8}(1 - e^{-a/0{\cdot}03})$. The observations of Boggess and Borgman[5] are shown as crosses; those of Stecher[6] as filled circles; those of Stecher[7] as triangles; and those of Bless and Savage (private communication) as open circles. The normalization is according to $\Delta m = 0$ at $\lambda^{-1} = 1{\cdot}8\ \mu\text{m}^{-1}$, $\Delta m = 1$ at $\lambda^{-1} = 2{\cdot}3\ \mu\text{m}^{-1}$.

It is concluded that a simple size distribution of diamond particles can be found which gives a calculated extinction curve satisfying the observational data as satisfactorily as any other model of interstellar grains so far proposed. It should be noted, however, that the existence of such a distribution does not constitute a strict test for the composition of the dust grains. A simple distribution which fits the extinction observations could probably be found for particles composed of many different materials.

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Mascons, Mare Rock and Isostasy

THE nature of the large positive gravity anomalies discovered on the Moon[1] (mascons) has been the subject of lively discussion. The high density (3·1 to 3·5 g cm^{-3}) of mare rocks at Tranquillity Base[2] supports one hypothesis—that mascons are surface sheets of high density volcanic rock[3]. The non-isostatic conditions of the mascon areas shown by the free-air anomalies[4,5] may have terrestrial analogues in some regions of recent volcanism where breaks in the crust have allowed dense basalts to pile up on the surface faster than isostatic balance can be maintained.

The form and magnitude of the mascon anomalies can be accounted for by sheets of mare volcanic rock denser than the rock of the adjacent highlands[3,5]. Should highland rock, which probably includes anorthosite of density 2·8–2·9 g cm^{-3} (ref. 6), prove to average less dense than mare rock by as much as 1 g cm^{-3}, then the large Mare Serenitatis and Mare Imbrium anomalies may represent mare sheets 10–15 km thick[3,5]. A third of this thickness would be unsupported isostatically, in inverse proportion to the excess density over highland rock. This imbalance would correspond to loads of the order of 200 bars.

If mare rock causes the anomalies, the question of why some maria have no anomalies may be answered in terms of the ages of the basins which they fill. The well identified mascons are all associated with relatively fresh and easily recognizable mare-filled circular basins: Imbrium, Serenitatis, Crisium, Nectaris and Humorum[1]. On the other hand, some of the mare areas without mascons, namely Tranquillitatis, Nubium and Fecunditatis, are in what appear to be degraded circular impact basins older than any of the mascon basins[7]. Thus only mare rock in the younger large impact basins seems to be isostatically unbalanced.

The maria themselves are younger than any of the large circular basins and were formed during a period of extensive volcanic flooding[7]. If all mare rocks are 2–4 × 10^9 yr old as reported for the Apollo 11 and 12 samples[8,9], then the non-isostatic condition has persisted for a long time during which the rigidity has been sufficient to support regional loads of the order of 200 bars.

I propose that before mare flooding the lunar rigidity was much less than after, so that considerable isostatic adjustment was possible before but not after. This sequence is suggested by a contrast in morphology between young and old craters. With rare exceptions most post-mare craters are deep although they are as much as 2–4 × 10^9 yr old, yet many pre-mare craters have shallow raised floors that indicate isostatic uplift[10]. Some of the large impact basins are old enough so that, by the time flooding started, substantial isostatic levelling would have already occurred. As a result, only a thin layer of mare material was deposited in these old basins. Younger basins had not yet reached equilibrium and were still deep by the time flooding started, so they were filled with a great thickness of mare material, in excess of the amount that would be isostatically balanced. By this time, the outer part of the Moon had cooled to a more rigid state so that isostatic adjustment could not take place.

Similar volcanic flooding on Earth apparently can also cause temporary imbalances, which rectify themselves through isostatic creep during prolonged periods of quiescence. Positive gravity anomalies characterize the young basalt provinces of Hawaii[11] and Snake River Plain[12], and some large regions of young oceanic flood basalts, notably the Iceland–North Atlantic area and the Kerguelen–Crozet area[11]. One interpretation of these regions is that relief of pressure by breaks in the crust, such as by sea-floor spreading, leads to inward lateral transfer of material in the asthenosphere. The resulting excess material piles up above its equilibrium level rather than spreading out because the crust in these areas is too cool to creep rapidly[11]. Why the excess crust does not sink into a flowing asthenosphere has not been explained; it seems clear, nevertheless, that the dynamics of volcanism keep these regions unbalanced. They contrast with areas where volcanism ended before the Tertiary and approximate isostatic equilibrium was restored by slow creep, and with areas such as the south-east Pacific, where heat flow is high and isostasy maintained despite current volcanism[11].

This brings us to a possible explanation for the apparent isostatic equilibrium of Oceanus Procellarum. This vast mare area may represent not merely volcanic flooding into a crustal scar, as in the case of the circular basins, but a major centre of high heat flow. It has already been suggested that three major volcanic plateaus in Oceanus Procellarum may hint at an origin similar to that of the terrestrial mid-ocean ridges[13]. Thus, by analogy with the south-east Pacific, the Procellarum region may have been warm enough to maintain isostasy during loading.

Reliable gravimetric data over most of the Moon are indispensable for assessing the various mascon explanations that have been proposed. It will be particularly exciting to discover whether gravity anomalies are associated with circular basins not flooded with mare, such as several on the far side or the basin near Schiller, or with very ancient flooded circular basins such as that occupied by Mare Australe[7]. The hypothesis proposed here predicts that such basins have no mascons.

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Apollo 11 Evidence for the Differentiation of Lunar Materials

THE first detailed chemical analysis of lunar rocks[1] has renewed interest in the problem of the origin of the Moon. Although many controversies revolve around this question, it is reasonable to begin with the hypothesis that the Moon was originally formed from a cloud of gas and dust approximating the solar or "cosmic" composition and that it underwent the obvious differentiation not only with respect to certain gases but also with respect to more refractory elements. Differentiation could have occurred within the hypothetical solar nebula itself even before separation of the condensed body, through ambipolar diffusion in a mixture of neutral and ionized gases in a magnetic field[2] or by differential magnetic forces on solids[3]. It could also have occurred during condensation and agglomeration into the condensed sphere and later through igneous and metamorphic processes. Discussions of possible differentiation through magmatic processes have already been given by Salisbury[4], O'Keefe and Cameron[5], Lowman[6] and Walter[7].

The chemical compositions of the rocks must also be considered in relation to the history of the entire surface. In general the data so far tend to substantiate Baldwin's thesis[8] that the surface features, including the circular maria, owe their existence chiefly to impact explosions of solid bodies varying in kinetic energy. An important corollary which follows from this thesis is that the lunar deposits have been strewn and restrewn across the surface and that they have been disturbed to depths of the order of many kilometres. The extent to which igneous and other processes have modified this picture of lateral and vertical mixing is as yet unknown but is subject to test by chemical analyses such as these discussed here.

A parameter which has been found useful in correlating certain geochemical data is the first ionization potential of an element[9]. Indeed, this parameter also enters directly into the theory of ambipolar diffusion and so should be directly related to possible differentiation in the preplanetary nebular gas[10]. Figs. 1 to 3 show plots against the ionization potentials of elemental abundances of a representative lunar sample[1], of the Earth's crust[11] and of the class I carbonaceous chondrites[12]. In each case the plots are normalized to the elemental abundances of the solar gas[12], and the abundance of silicon is assumed to be the same in all cases.

Perhaps the most significant feature of the chemical analyses is the great similarity of composition of all the rocks from Tranquillity Base. The uniform abundances of Ti, Fe, Mg, Ca and Na are especially marked. Another significant feature is the qualitative similarity between the distribution patterns of the lunar rocks and of the Earth's crust (Fig. 2). It is interesting that the parallel holds despite the high crustal abundances of elements with low ionization potentials.

The abundances of certain of the chief constituents such as Ti, Fe and Na differ greatly from their abundances in basalts, which are the most similar common terrestrial rocks in other respects[13]. Particularly anomalous in terms of terrestrial rocks is the combination of high values of the atomic fraction Fe/(Fe + Mg) and low Na. In terrestrial igneous differentiates Fe/(Fe + Mg) and the albite content usually increase together and this holds even when absolute iron enrichment occurs (my unpublished work).

The lack of resemblance between the lunar elementary abundance distributions and that of the class I carbonaceous chondrites (Fig. 3) is to be expected because the latter are usually regarded as the least differentiated condensed matter known from the solar system[14]. Comparison with the lunar patterns thus immediately indicates that the lunar materials have undergone some differentiation among the refractory elements.

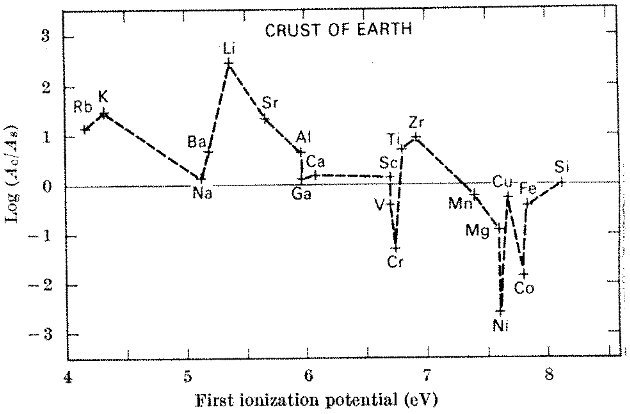

Fig. 2. Normalized logarithms of the elemental abundances in the Earth's crust[11] plotted against the first ionization potentials. A_c indicates the crustal elemental abundances and A_s indicates the solar elemental abundances to which the A_c values are normalized.

The rocks from Tranquillity Base have such high densities that even the vesicular rocks can be up to 3·4 g cm^{-3}. It seems likely that the true densities are even higher than this. The fact that these densities exceed the mean density of the Moon (3·34 g cm^{-3}) points to a differentiation process by which iron is preferentially concentrated in the outer layers. Indeed, the gravitational instability which such a situation implies has its parallel on Earth in the differentiated and layered gabbroic bodies such as the Skaergaard[15] complex of east Greenland and the Stillwater[16] complex of Montana. In the case of the Stillwater complex[16] the fractionation of Fe^{2+} and Mg between the earliest melt, as represented by rocks of a quenched border facies and the olivine crystals precipitated initially, is such that iron is highly concentrated in the melt relative to Mg. Similar relations also apply to the Skaergaard complex[16], so that in the terrestrial differentiated complexes analogous to the lunar rocks, iron is highly concentrated in the melt relative to magnesium as crystallization proceeds. The Apollo 11 data also point to a similar relation for the rocks at Tranquillity Base if the fractionation is estimated from the olivine phenocrysts and the rock as a whole.

It has been shown experimentally[17] that crystallization in the system MgO–FeO–Fe$_2$O$_3$–SiO$_2$ under anhydrous and reducing conditions leads both to an increase of the Fe^{2+}/Mg^{2+} ratio in the melt and to absolute iron enrichment. Operation of the same mechanism in the gabbroic complexes leads to a compositional layering in which the density increases upward in the stratigraphic sequence[15]. Thus the high densities of the lunar surface rocks are not surprising because a partial melting of the lunar layers by any of a number of mechanisms could result in a melt enriched in iron relative to the unmelted residue. Such a melt might well have a density lower than the surrounding solid rock due to considerable expansion on melting[18], so that it would move to the surface under buoyant forces.

An important difference between the terrestrial and lunar rocks is the low water content of the latter indicated by the lack of hydrous phases. Water has the effect of increasing the solubilities of the feldspars relative to those of the ferromagnesian minerals in the melt so that under high water pressures, such as are attainable on Earth, the melt and the crystallization products tend to be feldspathic[13]. On Earth this process seems to give rise to large volumes of rock belonging to the granite–diorite series which form the bulk of the continental crust and which are reflected in Fig. 2. As previously noted, the lunar rocks seem to be more like the relatively anhydrous

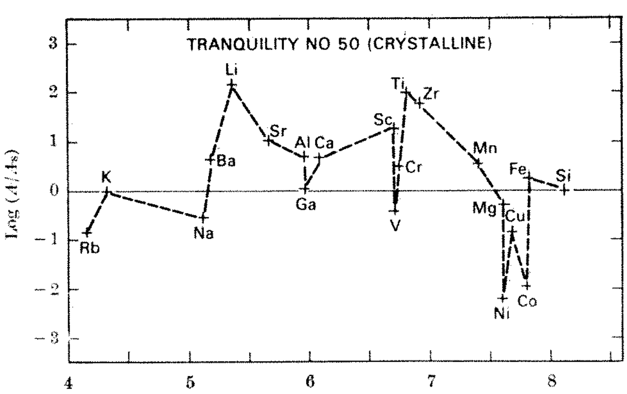

Fig. 1. Normalized logarithms of the elemental abundances of a representative lunar rock type[1] from Tranquillity Base plotted against the first ionization potentials. A indicates the lunar elemental abundances, and A_s indicates the elemental abundances to which the A values are normalized. The size of the crosses corresponds to 10 per cent uncertainty in the chemical analyses.

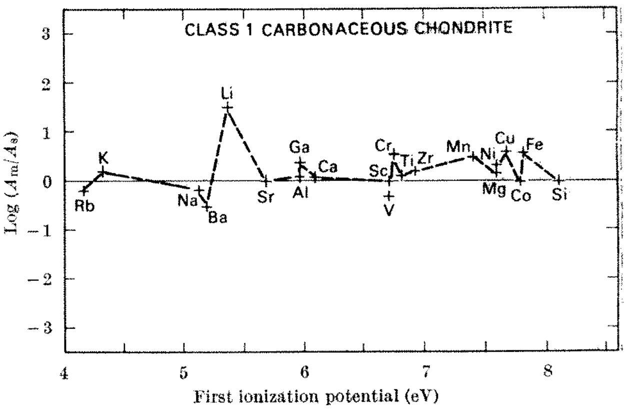

Fig. 3. Normalized logarithms of the elemental abundances of the class I carbonaceous chondrites[12] plotted against the first ionization potentials. A_m indicates the meteorite abundances and A_s indicates the solar elemental abundances to which the A_m values are normalized.

terrestrial gabbros because they contain large amounts of ferromagnesian minerals. Walter[7] has already called attention to an analogous effect of low water pressure on the melting properties of the lunar rocks.

The high TiO_2 contents of the specimens from Tranquillity Base can be accounted for in part by magmatic differentiation of the gabbroic type, as this oxide has been observed to increase upward in the Skaergaard complex[15]. It is possible that concentration of TiO_2 in the melt is even more favoured under conditions of low oxygen fugacity which would inhibit its precipitation as titaniferous magnetite. It is also possible that a relatively small initial difference between the magmatic source rocks of Tranquillity Base and those of terrestrial basalts could be amplified by differentiation.

In summary, the compositions of the surface rock at Tranquillity Base seem to be consistent with a differentiation mechanism, perhaps of a complex character embodying igneous and other processes, but which probably contained elements similar to that which produced the crust of the Earth. There are important modifications induced by the low water content of the lunar rocks, however.

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Surface Temperature of the Early Earth and the Nature of the Terrestrial Atmosphere

THE detailed models of solar evolution now available clearly indicate that the luminosity of the Sun has been gradually increasing since it first arrived on the main sequence some 5×10^9 yr ago. If it is assumed that any changes in the orbit of the Earth during this time have been periodic—so that the present orbit is very nearly the average orbit—then it follows that the value of the solar constant has also increased correspondingly. The possibility of a resultant variation in the surface temperatures of the planets is of particular interest for the Earth, which is the only planet at present possessing a considerable quantity of water in the liquid state on its surface.

If we accept the view that the Earth's atmosphere has accumulated by gradual degassing of the crust, we must suppose that the Earth was originally devoid of atmosphere. In this case, the Earth's albedo would have been low initially, increasing gradually as a thin atmosphere formed. Taking 0·15 as a representative figure for the albedo of the early Earth, we can estimate from solar model calculations[1] that the average initial surface temperature of the Earth would be about 245 K. On the other hand, the local midday temperature might have been as much as 100° higher than this. As a result, any water vapour degassed would be predominantly frozen out on the surface, though local heating would return some to the atmosphere. (In the initial stages of atmospheric development the pressure would, of course, be too low for the formation of liquid water.)

This estimate of the surface temperature supposes, however, that the atmospheric gases contributed no greenhouse effect. Whether this is true or not will depend on their composition. There are two possibilities here. The first is that from early on some of the compounds degassed were oxidized (in particular, that an appreciable amount of carbon dioxide was present). The second is that the compounds were originally all reduced (methane and ammonia being especially important).

It is well known that carbon dioxide in sufficient quantity can produce a marked greenhouse effect. A rough calculation suggests, in fact, that a maximum of 200 g cm^{-2} of atmospheric carbon dioxide would be required to raise the average surface temperature of the Earth from the initial value of 245 K to above the freezing point of water. This estimate supposes, however, that the carbon dioxide acts alone; but actually, as the temperature rises, so the amount of water vapour in the atmosphere will also increase. Water vapour is an efficient infrared absorber, but has a window in the 9–18 μm region where the terrestrial radiation peaks. Carbon dioxide, on the other hand, has a main absorption band in this region. The result is that the two gases acting together produce an enhanced greenhouse effect as compared with either acting alone. Hence the figure quoted for the required amount of carbon dioxide is a considerable over-estimate; after all, the present Earth has a greenhouse effect $\sim 35^\circ$ with only 0·5 g cm^{-2} of carbon dioxide.

If we turn now to the comparable situation in a reducing atmosphere, we find that ammonia also absorbs within the water vapour window. Hence a combination of ammonia and water vapour, like carbon dioxide and water vapour, would produce an appreciable greenhouse effect. A reducing atmosphere could therefore raise the surface temperature of the early Earth in the same way as an oxidizing atmosphere. The difference is that ammonia is much more soluble than carbon dioxide in water. As a result, any extensive formation of liquid water on the Earth's surface would lead to a rapid removal of almost all atmospheric ammonia. If we suppose that all the nitrogen currently present in the atmosphere and sediments has been derived from degassed ammonia (obviously an upper limit), and that all the water vapour which has been degassed is now collected in the oceans (a lower limit), then the ratio of degassed ammonia to water vapour is certainly less than 1 per cent. This is insufficient to depress the freezing point of water by any great amount. Thus, the removal of ammonia from a reducing atmosphere by solution in water would lead to a loss of the greenhouse effect and to a refreezing of the water. (In fact, there is no need for the water ever to be present as such—ammonia can be absorbed directly by ice[2].) Hence a reducing atmosphere would keep the Earth's temperature constant at, or below, the freezing point of water.

From the amount of carbon dioxide currently present in carbonate deposits, it might be expected that a carbon dioxide atmosphere would accumulate around the Earth rapidly. For example, if carbon dioxide accumulated at a steady rate, the figure of 200 g cm^{-2} would be reached in $\sim 10^7$ yr. It follows from the foregoing argument that, with a carbon dioxide atmosphere, liquid water should have appeared on the Earth's surface very early in its history. This should not be true for a reducing atmosphere. One possible method of checking on the chemical composition of the early atmosphere, therefore, is by examining the early geological record for effects attributable to water. An oxidizing atmosphere should produce indications of erosive activity, whereas a reducing atmosphere should not.

In fact, some of the oldest rocks known include metamorphosed sediments. It follows that there must have been active erosion well before $3 \cdot 5 \times 10^9$ yr ago. The Earth should have had an oxidizing atmosphere for at least this length of time.

There are two points to be noted about this argument. The first is that, if liquid water is created in the presence of excess carbon dioxide, it might be expected that some of the latter would be removed by the Urey reaction[3]. Not only would this reduce the greenhouse effect, but the resultant SiO_2 deposits might be expected to appear in the geological record. It is certainly true that the growing oceans would stabilize the amount of atmospheric carbon dioxide. As long as the early oceans had the same character as the present ones, however, an adequate greenhouse effect would be produced by the carbon dioxide remaining in the atmosphere. As far as the deposition of SiO_2 is concerned, it must be remembered that the Urey reaction is really a symbolic representation of a more complex series of processes, some of which would certainly have gone more slowly on the primitive Earth. The situation seems to be that only partial pressures of carbon dioxide equivalent to one atmosphere pressure, or more, can be ruled out.

The second point to note, however, is that the complex buffering system of the oceans with respect to carbon dioxide, and, ultimately, the Urey reaction, do act as a control on the amount of carbon dioxide in the atmosphere. The presence of liquid water on Earth thus limits the extent of the greenhouse effect. It has been supposed in the foregoing discussion that the Earth degassed at a uniform rate. This, however, is rather improbable; it is much more likely that the initial emission of gases occurred more rapidly[4]. In this case, if a large amount of carbon dioxide degassed in the initial burst, the resulting greenhouse effect might produce a surface temperature which immediately exceeded the boiling point of water. With no liquid water present, there would be no mechanism capable of removing carbon dioxide rapidly from the atmosphere. The surface temperature would remain high, and no oceans would form. This has obviously not occurred on Earth, but it may describe what happened on Venus, where, in any case, the initial surface temperature must have been $\sim 50°$ higher due to the greater proximity of the planet to the Sun.

I thank several of my colleagues in the Geology Department for their advice on various aspects of this work.

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New Model for the Structure of the Ocean Crust

Using the ocean floor spreading/plate tectonics theory as a starting point, examining the implications of this theory for processes taking place at mid-ocean ridge crests, and adding data collected from dredged rocks and geophysical measurements in the oceans, it is possible to build up a well controlled model of the structure of the ocean crust and the way in which it is formed.

Studies of magnetic anomalies[1,2] and earthquake fault plane solutions[3,4] have shown that the mid-ocean ridges are lines along which plates of the lithosphere are moving apart. During this movement, upper mantle material rises as a hot diapiric intrusion from the mobile asthenosphere to fill the crack between the plates. Material from within the asthenosphere will be hot enough, if it rises fast and cools essentially adiabatically, to pass through the basalt melting curve and form an interstitial basaltic melt[5]. (This is not to imply that an interstitial melt is not present in the asthenosphere, but only that its composition is not basaltic.) The formation of such a basaltic interstitial liquid is demonstrated by the eruption of basalt lavas all along the crests of the mid-ocean ridges. Evidence from magnetic anomalies[6,7] and petrography[8] shows that the zone of eruption of lavas is very narrow, certainly less than 25 km wide, and perhaps in places as narrow as 0·5 km. This basaltic material must form at least a large part of the ocean crust[5], and the formation of new crust must thus be confined to a narrow strip along the mid-ocean ridge crests.

It is important in this analysis that the incoming mantle material must rise fast from 100–200 km depth along a narrow zone. Thus the widening crack between the lithospheric plates must be narrow and nearly vertically sided. Models with gently sloping sides to the lithospheric crack, attractive on other grounds because of the lower velocity necessary in the rising mantle, do not seem to fit the requirements of melting and eruption (Fig. 1).

What, then, accounts for the transition on the walls of the crack from mobile asthenospheric material to rigid lithospheric material? In such a vertical-sided crack, the explanation cannot be, as it could be in a crack with a tent-like shape, the cooling of the rising material by conduction through some temperature interval separating plastic from rigid behaviour. Rather it must be that the mobile behaviour is associated with interstitial liquid, which not only lubricates grain contacts but acts as an ionic carrier during the recrystallization that must accompany flow. Thus as the mantle material rises it flows more and more readily because the amount of interstitial liquid increases with progressive melting. When the liquid has increased in volume enough to leave its parent material and rise further towards the surface, however, the ability of the parent material to flow is drastically reduced, and it becomes at this stage part of the lithosphere.

Although much of the rising asthenosphere loses its liquid at some depth in the crack, part at least of the material must retain its interstitial liquid while it flows to near the top of the crack in the lithosphere to form the mantle immediately beneath the Mohorovičić discontinuity. To reach this position the material must have retained its high temperature or the interstitial liquid would solidify and increase the rigidity of the rising diapir. Thus the temperature at the level of the top of the mantle must be at about the melting point of basaltic magma (*c.* 1,100° C). Basaltic liquid rising from lower in the crack through this zone will not solidify there, but will accumulate as a pool of liquid overlaying the top of the column of asthenospheric material filling the crack. Thus, along the crestal zone of the mid-ocean ridge system, the lower part of the oceanic crust should consist of a magma chamber filled with basaltic liquid (Fig. 2).

Dredging from the crests of mid-ocean ridges shows that the basaltic liquid is usually erupted in the form of pillow lavas (sometimes it is found as thin ropy lava flows). The pillows form steep-sided hills, mushroom-shaped in cross-section, elongated parallel to the mid-ocean ridge axis. These hills form a capping on the vertical dykes that feed them. As cracking of the lithospheric plate continues, further eruptions of pillow piles take place, overlapping and building up on earlier ones, so that an upper crustal section of piled-up overlapping mushrooms develops.

In an earlier examination of processes at mid-ocean ridge crests[5], it was assumed that this piling up process continued until the whole ocean crust was composed of superimposed pillow lava piles. This is not, however, tenable, as no model can be constructed in which a pillow pile, originally at the top of the crust, becomes buried until it lies in contact with the mantle. Only a limited amount of burial can take place in practice, resulting in an upper skin to the crust of piled up pillow lavas. The thickness of this skin will depend on the ratio of extruded lava to that remaining in the dykes.

Beneath this skin is a zone made of basalt that has never reached the surface. This takes the form of a dense complex of feeder dykes, intruding each other and following the gaps between previous dykes, as was envisaged in the original models for magnetic anomalies[6,10]. These dykes rise from the magma chamber at the base of the crust. Near the magma chamber, where the ambient temperature is high and the rate of cooling slow, they will crystallize to coarse-grained rocks. Higher up, the rate of cooling will be faster and the grain-size correspondingly less, giving rise to basalts and dolerites.

In the active crestal zones of the mid-ocean ridges the crust consists of a layer of pillow lava units fed by dykes overlying a zone of dykes without lavas, which become coarser-grained downward towards a basaltic magma chamber at the base of the crust (Fig. 2).

Three metamorphic facies have been recognized in rocks from the ocean floor, a facies in which clay minerals develop from ferro-magnesian minerals of basalts[11], a greenschist facies[11,12], and a facies in which green hornblende replaces basaltic ferro-magnesian minerals[13]. The first two facies indicate the presence of abundant water during metamorphism, but the third, in which plagioclase shows no sign of reaction to more sodic compositions with the formation of epidote, apparently represents a higher temperature, but water deficient, environment.

Water in the ocean crust has two sources: sea water trapped in the cavities within and between the lava pillows as they form, and water from cooling and crystallizing basaltic liquids. Because of the low water content of basaltic liquids, the second source must be much less important than the first, and metamorphism within the layer of pillow lavas will take place under much more water rich conditions than within the complex of dykes that underlies it. The first two facies can thus be assigned to the pillow lava layer of the crust, and the third to the dyke swarm.

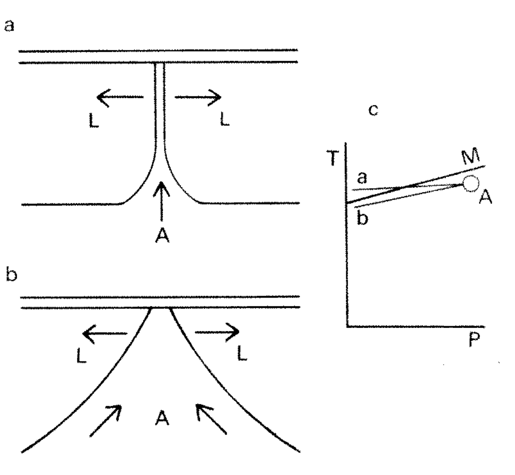

Fig. 1. L, Lithosphere; A, asthenosphere. *a* and *b*, Alternative models showing the lithospheric crack with vertical and sloping sides. *c*, Schematic P–T diagram showing how mantle material rising vertically (as in model *a*) from the asthenosphere (A) crosses the basalt melting curve (M) while mantle material rising slowly (as in model *b*) cools by conduction and does not cross the basalt melting curve.

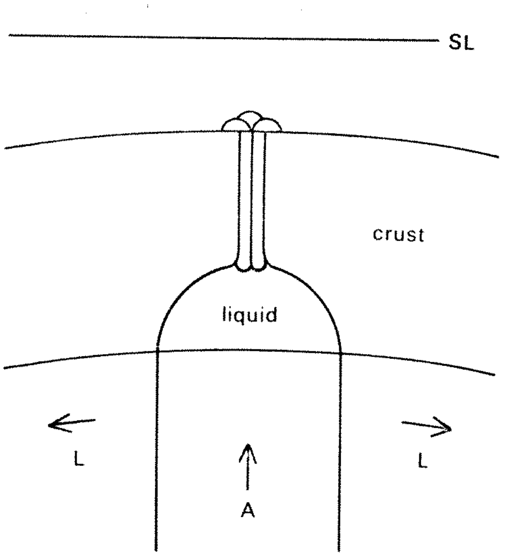

Fig. 2. L, Lithosphere; A, asthenosphere. An enlarged view of the crust and the top of the rising column under mid-ocean ridge crests, showing the active structures.

As the ocean crust migrates away from the ridge crest down the flanks of the ridge, some modifications of the structure in the active zone take place. First, the liquid magma chamber at the base of the crust slowly solidifies, losing its heat by conduction through the overlying ocean crust. During this process fractional crystallization will take place, with the formation of layered gabbros on the floor of the chamber, and with the production of the granitic or dioritic late differentiates that have recently been dredged from the oceans[14,15]. Water released during crystallization may help to cause localized metamorphism of the gabbros themselves[13], but some must also react with the underlying mantle, as the temperature falls, to form serpentinite. Because of its low density, this serpentinite will intrude upwards as diapiric masses through the ocean crust[13], giving rise to the serpentinites that have been dredged from the ocean floor.

The final crustal structure envisaged is shown in a somewhat diagrammatic form in Fig. 3 (the normal faults cutting the ocean crust have been omitted for clarity).

A zone of unmetamorphosed pillow lavas overlies metamorphosed pillow lavas in two zones. These are intruded by feeder dykes for the flows. Beneath this is the dyke swarm proper (mostly metamorphosed to the hornblende-bearing facies) which grades downwards through coarser-grained dykes to a massive layered gabbro forming the lower crust. Serpentinite diapirs from the uppermost part of the upper mantle intrude through this complex.

The most important consequence of this model is the postulated presence of a liquid lower crust along the length of the axial zone of the mid-ocean ridge system. The presence of this feature should be detectable by seismic or magneto-telluric means. The boundary between seismic layers 2 and 3 in the ocean crust is interpreted here as the junction between the zone of pillow lavas, metamorphosed in part, and the underlying more massive dyke swarm and gabbro, while the Mohorovičić discontinuity is the boundary between gabbro and peridotite. The heat flow at mid-ocean ridge crests is, in the model, controlled by the presence of the basaltic magma chamber in the lower crust. Assuming a depth to the top of this chamber of 4 km below the ocean floor, a temperature

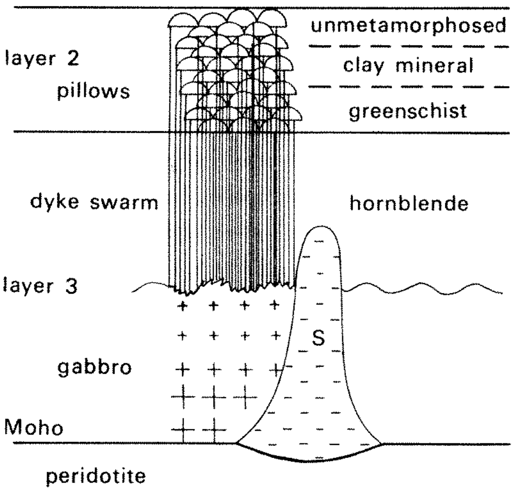

Fig. 3. Section through the ocean crust as envisaged by this model. S is a serpentinite diapir.

within the chamber of 1,100° C, and thermal conductivity of the overlying rocks as 4×10^{-3} calories^{-1} s^{1} degree^{-1}, a heat flow of 10×10^{-6} calories cm^{-2} s^{-1} may be calculated. This is within but at the upper limit of values measured near ridge crests. This agreement is satisfactory. Agreement with magnetic models of ocean crust structure is also satisfactory. Magnetic rocks will be found in the upper parts of the zone of pillows and in the dyke swarm metamorphosed under water deficient conditions. The layered gabbro composing the lower crust would be expected to be essentially non-magnetic, as would also be the basalts metamorphosed in the greenschist facies.

The model has been constructed entirely from the consequences of the plate tectonics theory and from consideration of the rocks and geophysical measurements from mid-ocean ridges. No reference was made, or was necessary, to the ophiolite complexes seen on the continents, which many people interpret as slices of ocean crust thrust in among continental rocks during mountain building[16,17]. When comparison is made between the model and the observed structure of ophiolite complexes, such as the Troodos complex in Cyprus[17] and the South Oman complex in Arabia[18], the similarities are consequently very striking. Both of these ophiolite complexes can be interpreted as consisting of exactly the same elements as the model, gabbro overlying peridotite and itself overlain by an intense dyke swarm, which in turn is capped by a layer of pillows.

The fact that complexes very like the model exist reinforces both the model and the interpretation of ophiolite complexes as pieces of ocean crust. Reference to the structure of the ophiolites enables refinements to be made to the model, for example that the contact of the gabbros with the peridotites is rather complex, with gabbro dykes in the peridotite and peridotite xenoliths in the gabbros. This contact zone was measured as about 500 m thick in the South Oman complex[18], a thickness compatible with geophysical observations of the oceanic Mohorovičić discontinuity. On the other hand, reference to the model allows interpretation of features seen in ophiolite belts. The association of gabbro with peridotite can be seen to be genetic, and the association is explained. In addition, the recognition that the sub-oceanic Mohorovičić discontinuity outcrops in ophiolite belts allows this boundary to be studied without the need for expensive drilling operations.

I thank the Sedgwick Club for stimulating me to think along these lines.

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Sub-Sea Floor Structure south of France

It has been suggested[1,2] that the Ligurian Sea was formed by the Corsica–Sardinia block drifting away from the south of Provence, during the Oligocene or the Miocene. These suggestions take no account of sub-sea floor structures.

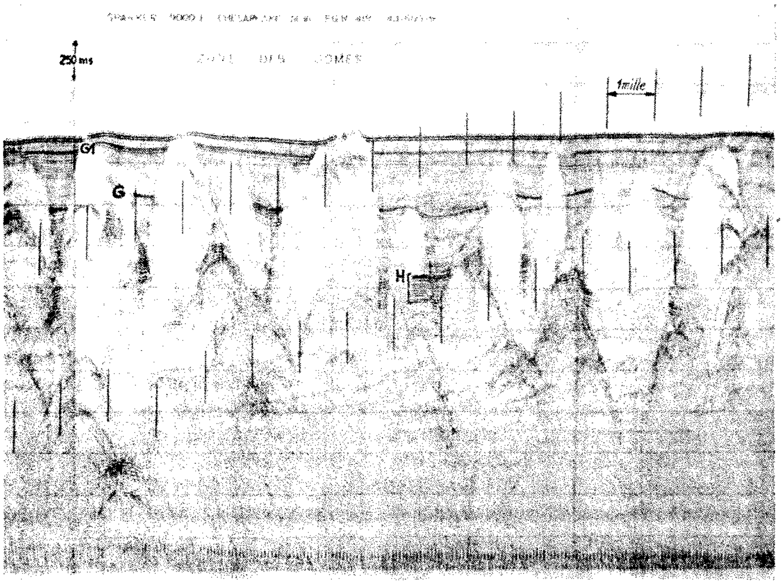

Fig. 1. *H* reflector in *A* zone: this reflector appears as a sequence of about 250 ms lying about 1 s (± 200 ms) below the sea bottom (water depth = 2,600 m). *N.B.* All times are double way times. All sections E.G. & G. sparker 9,000 J, Chesapeake M-16 hydrophone and PGR Alden 419 or E.G. & G. 254 recorders. Ship's velocity was about 6 knots—vertical scale, between scale lines 1 second on E.G. & G. 254 and 250 ms on PGR Alden 419.

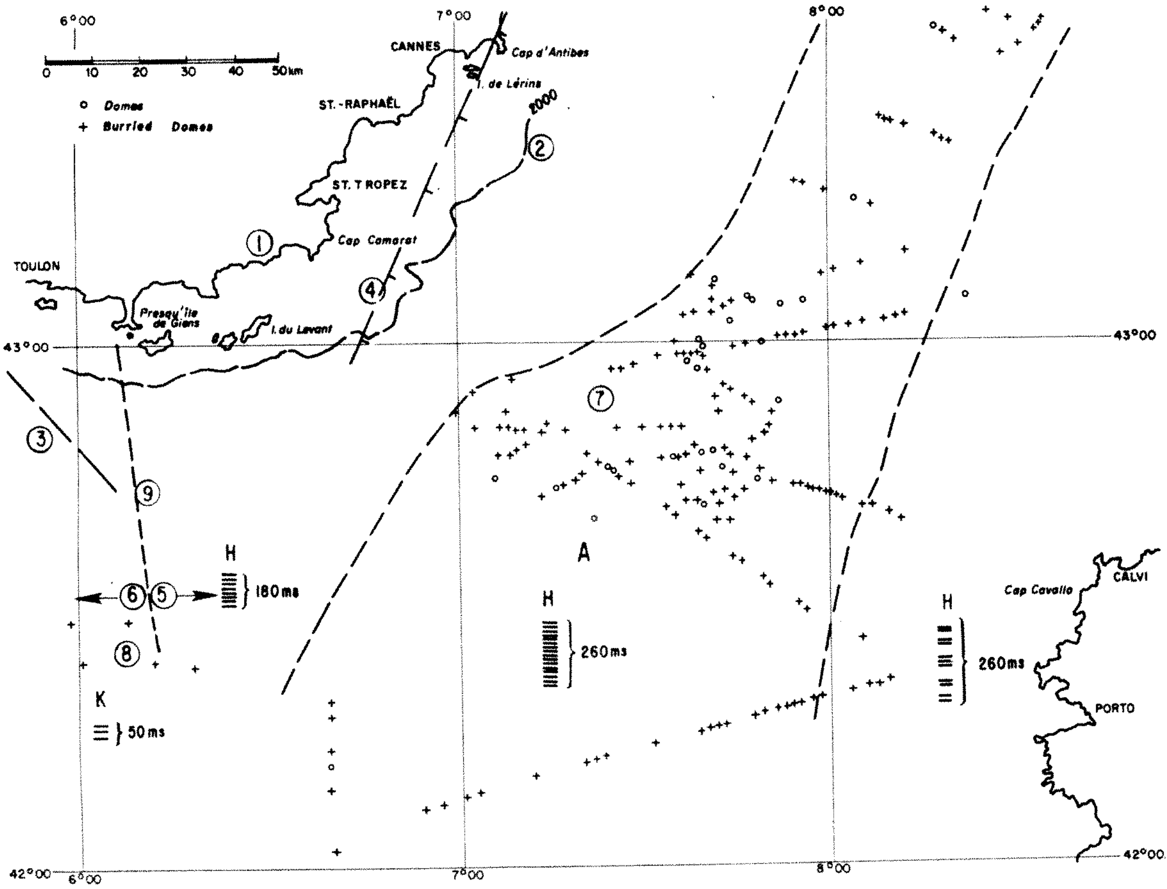

Fig. 2. Geographical distribution of the reflectors with the major accidents of the region: 1, coast line; 2, slope; 3–4, probable extension of Arles and Antibes faults; 5, presence of *H*, and 6, *K* reflexion's areas; 7, a zone with typical salt domes; 8, domes found south of Toulon; 9, zone of complicated tectonics. No reflectors can be followed for more than a few miles.

The deepest reflector in the north-west Mediterranean, as shown by continuous seismic profiling in the *A* zone of Glangeaud[3], is the *H* level[3]. This seems to be more a sequence of reflexions than a single response[5] (Figs. 1 and 2). Its character changes east of the *A* zone, where the domes become smaller and thinner[6] and disappear towards Corsica. Westward, out of the *A* zone, it shows numerous diffraction patterns. South of Saint-Tropez, this *H* level becomes shallower, and erosion patterns can be seen near the continental slope where a deeper reflector, *D*, can be traced (Fig. 3). South of Toulon, we found domes[7] larger than those of the *A* zone and comparable with the Sigsbee knolls[8] (Fig. 4).

The deepest reflector west of the *A* zone is called the *K* level[5]. Its depth is about the same as the *H* level, but it is distinctly different in character (Fig. 5), although the sedimentary section presents the same features. The southern extension of those horizons is not yet well established. Their geographical distribution is schematically shown in Fig. 2.

The various characters of the deep reflector which lies below the abyssal plain south of France seem to be related to the different palaeogeographic areas. A first interpretation has been given by Glangeaud[9]. Fahlquist and Hersey[10] computed a layer with a compressional velocity greater than 4 km/s, about 1·5 km below the abyssal plain. Should this be interpreted as a salt bed or is it connected to the magnetic basement which has been found by a local study at about the same depth[11]? There are, as yet, insufficient data to lead to a certain conclusion, but we may correlate Fahlquist's layer with the reflector *D* (see Fig. 3).

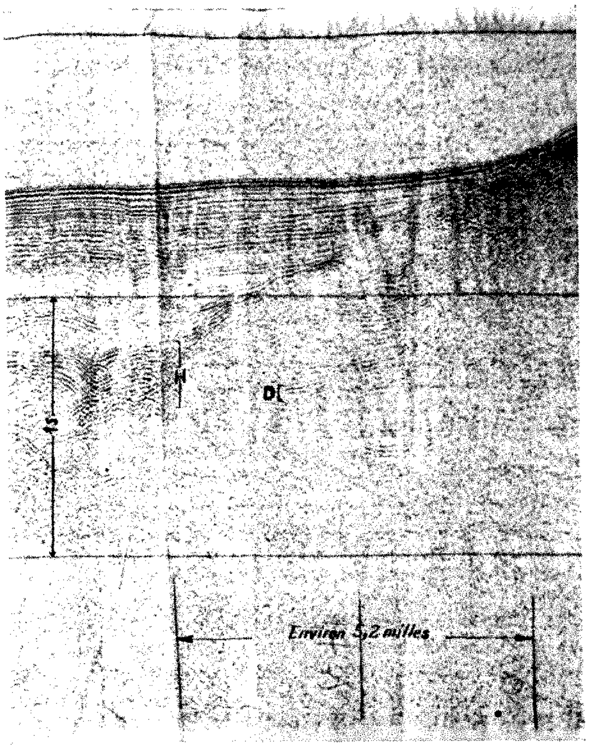

Fig. 3. Sparker record on the continental rise south-east of Levant Island (water depth = 2,400 m). Reflector *H*, and below it, *D*.

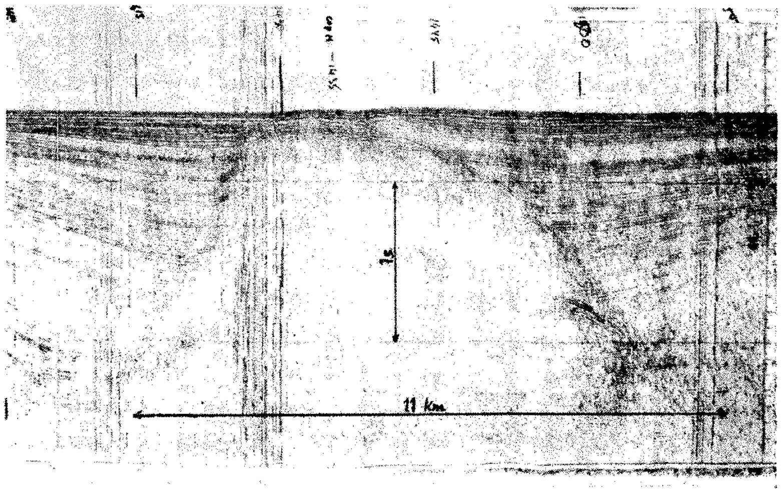

Fig. 4. Typical section of a dome south of Toulon (water depth = 2,400 m).

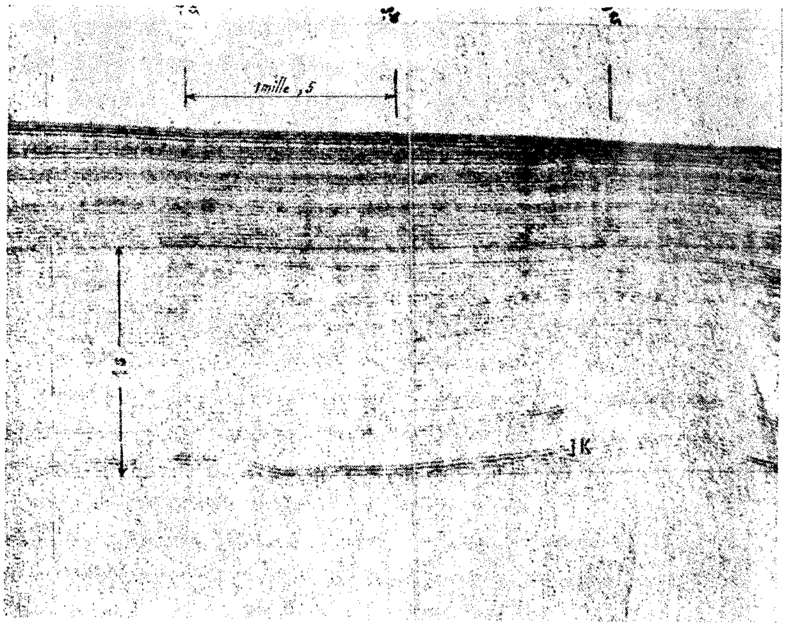

Fig. 5. *K* reflector (west of 06° 00′ E). *K* reflector itself is formed by three phases. The lowest one is corrugated; *K* reflector stays at about 180 ms below a double phase reflexion (water depth = 2,400 m).

Magnetic data therefore indicate Tertiary volcanic deposits such as those found on land, the salt evidently coming from below the *H* horizon (see Fig. 1). The depth of the recorded reflectors, the erosion evidence that can be seen on the *H* level and the probability that there is a deeper level lead to the assumption—following Glangeaud's hypothesis[9]—that this salt basin was formed before the Oligocene or Miocene, as proposed by Stanley and Mutti[1].

Profiling should improve our knowledge of the abyssal structure. Coring from 50 to 100 m under the sea floor will be needed to prove the existence of salt. To determine the date of the *H* and *K* levels, and thus of the entire basin, drilling should be carried out to at least 400 m below the sea floor.

The survey was carried out under contract with the Centre National pour l'Exploitation des Océans and with the help of the Research Committee of the National Geographic Society and the Campagnes Océanographiques Françaises.

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Rotation of Sardinia: Palaeomagnetic Evidence from Permian Rocks

PALAEOMAGNETIC data from Middle Tertiary rocks have not decisively shown any rotation of Sardinia[1]. Rocks of Permian age, which seemed most suitable for detecting rotations of the Southern Alps[2,3], Corsica[4] and the Iberian Peninsula[5], were therefore collected in Sardinia in the hope of finding more conclusive evidence.

The samples were collected from several Permian rhyolitic ignimbrites and red sandstones in north-western Sardinia (Fig. 1). The local geology is described elsewhere[6-8].

Laboratory measurements of the rock samples revealed NRM directions with south-easterly declinations and small inclinations. All samples were examined by routine demagnetization techniques with alternating fields up to 3,000 oersteds. With the ignimbrites, alternating fields in general of less than 400 oersteds eliminated small secondary components, resulting in slight adjustments of the directions initially measured. In nearly all ignimbrite samples the total NRM could be practically eliminated at 3,000 oersteds—a typical example is given in Fig. 2*a*. Only in the samples of site 3 did an appreciable part of the NRM (with its characteristic direction) remain even after treatment at 3,000 oersteds (Fig. 2*b*).

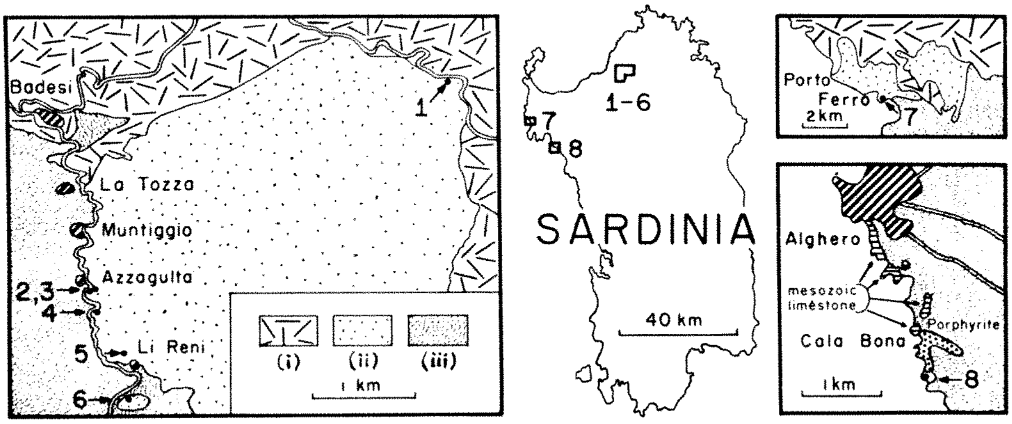

Fig. 1. Simplified geological maps of the sampling regions (after Traversa[7] and Moretti *et al.*[8]) with sampling sites. Key to hatchings: (i) rock basement below Permian volcanics; (ii) Permian volcanics and sediments; (iii) younger volcanics and sediments.

The alternating field demagnetizations of the red sandstones of site 7 showed that their NRM is composed of a very hard characteristic component and a large and fairly hard secondary component. Although the secondary magnetizations of the sandstones could be substantially reduced with the smaller alternating fields, it seemed that a complete elimination could only be achieved with alternating fields as large as 2,000–3,000 oersteds (Fig. 2*c*). The very hard characteristic component was only slightly reduced after treatment in the large alternating fields. The NRM of the red sandstones of site 8 seems to consist only of the very hard characteristic magnetization (Fig. 2*d*). A few thermal demagnetizations of these redbeds yielded results consistent with those obtained from the alternating field demagnetizations. The dispersion of the NRM directions of the red sandstones becomes less after correction for the tectonic tilt.

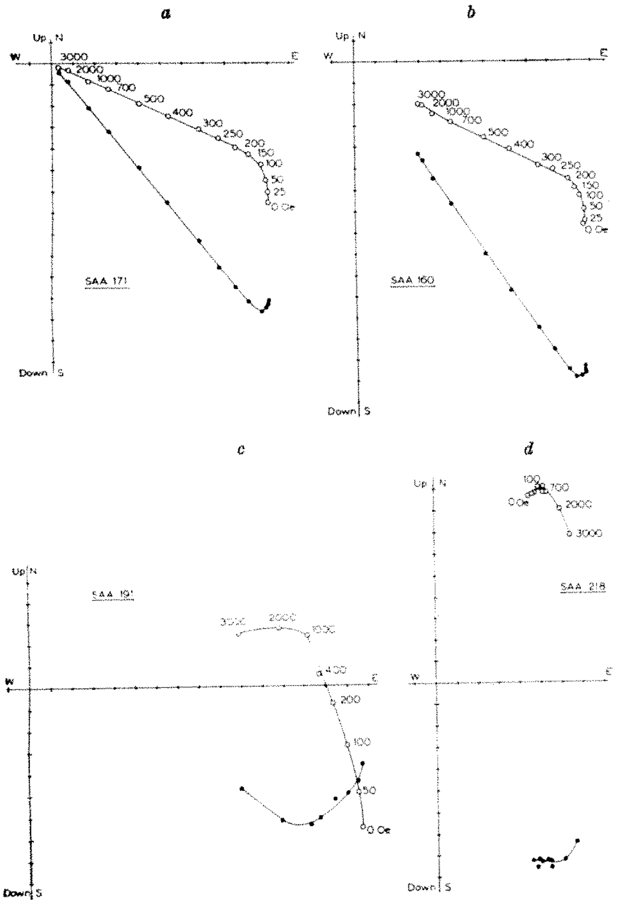

Fig. 2. Four examples of pilot sample demagnetization diagrams, from the rhyolitic ignimbrites (*a* and *b*) and the red sandstones (*c* and *d*). The points represent orthogonal projections of the successive positions of the terminus of the resultant magnetization vector during progressive demagnetization by a.c. magnetic fields. The numbers denote the intensities of these fields in oersteds. Open symbols indicate projections on the east–west vertical plane; full symbols indicate projections on the horizontal plane.

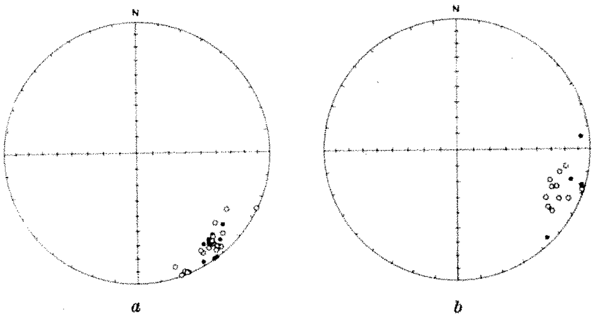

Fig. 3. Directions of magnetizations from the Permian rhyolitic ignimbrites (*a*) and the Permian red sandstones (*b*) after partial demagnetization in a.c. magnetic fields up to 3,000 oersteds and with respect to the bedding planes. Open (full) symbols denote north-seeking poles pointing up (down).

Table 1. SUMMARY OF PERMIAN DATA FROM SARDINIA

Site	Rock type	Bedding plane	J/cm^3 × 10^6	Q	Samples used/taken	Mean characteristic remanent magnetization direction With respect to present horizontal D	I	K	With respect to bedding α_{95}	D	I
	Rhyolitic ignimbrites										
1		109–16	330–480	1·3–3·0	8/8	140	−1·5	74	6·5	138·5	−9·5
2		312–12	15–55	0·7–1·8	3/6	141	−8	26	24·5	142·5	−6
3		12–10	16–80	0·8–3·8	5/6	143	−4	503	3·4	143·5	−11·5
4		7–8	75–190	2·7–5·0	4/4	129·5	+10·5	27	18·0	129	+3·5
5		7–8	60–90	3·5–4·5	5/5	159	+1	454	3·6	159	−2·5
6		7–8	75–175	3·0–9·0	4/5	143	+21	733	3·4	141·5	+15·5
	Redbeds										
7		Varying	2–4	0·5–1·4	11/15	112	+15	15	12·2	112	−7·5
8		133–46	2–4	1·7–3·3	4/5	135	−34	293	5·4	108	−24
Mean direction of magnetization of the rhyolitic ignimbrites ($N = 6$)								34	11·5	142·5	−2
Mean direction of magnetization of the red sandstones ($N = 2$)								—	—	110	−15·5

Table 2. PERMIAN POLE POSITIONS

	Lat.	Long.
Stable Europe, mean	47° S	21° W
Sardinia, ignimbrites	38° S	59° E
Sardinia, redbeds	20° S	91° E
Africa, K3 Galula redbeds	46° S	40° E
Africa, K3 Ketewaka–Songwe redbeds	27° S	89° E

A comparison of the palaeomagnetic results of Sardinia with the only two Permian data available for the African continent reveals a remarkable conformity. The apparent pole position for the ignimbrites of Sardinia is situated halfway between the pole positions of the K3 Galula redbeds and the Ketewaka–Songwe redbeds of Tanganyika, and the apparent pole position for the redbeds of Sardinia is very close to the Ketewaka–Songwe pole position. Because of the restricted number of palaeomagnetic data of Sardinia as well as of Africa, however, a definite conclusion from their conformity seems to be premature.

The mean inclination values of both the Sardinian ignimbrites and the redbeds fit in well with the palaeomagnetic field pattern, extrapolated for the Sardinian region from Permian palaeomagnetic data of the European continent. The mean palaeomagnetic declination values found in the Permian rocks of Sardinia, on the other hand, deviate considerably from the declination value, extrapolated from Permian data of stable Europe. This difference is attributed to a counterclockwise rotation of Sardinia, over approximately 50° in post-Permian times, with respect to stable Europe.

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Palaeolatitudes from JOIDES Deep Sea Sediment Cores

IN the framework of plate tectonics the Earth is thought to be composed of a mosaic of rigid plates riding on top of a viscous asthenosphere. The motion of these plates with respect to the Earth's spin axis can be determined using the methods of palaeomagnetism. Measurement of continental rocks[1], oceanic islands[2] and on seamounts[3,4] all yield data which can be used to determine these motions.

Palaeomagnetic measurements of deep sea sediment cores could yield additional information about the past motions of the sea floor. Because of their short length of less than 20 m, however, these cores have been studied principally to determine the frequency and pattern of reversals in the Earth's field during the past 5–10 m.y. With the advent of the JOIDES deep sea drilling programme, cores are now available spanning most of the depositional history of the sea floor.

During Leg II of the JOIDES deep sea drilling programme[5], on station 10 (32° 52′ N, 52° 13′ W), core was recovered spanning a large enough time sequence to enable a reasonable statistical analysis to be made of the palaeomagnetic data. Twenty cores were taken in all and covered two main sequences; the first seven, the Miocene through Upper Eocene, and the last ten, almost the entire Upper Cretaceous (Campanian). Two intermediate cores sampled the Middle Eocene and the Upper Maastrichtian. Opdyke[6] has previously discussed the sequence of polarity reversals recorded in this core. Here we discuss the record of changes in inclination.

The cores on which successful palaeomagnetic measurements were made separate naturally into five groups. Though the relative declination and intensity of the samples vary considerably, the five groups after a.c. demagnetization in a field of 50 oersteds possess absolute values of inclination which are consistent within each group but different between groups. The mean value for for each group, neglecting core 5, for which the intensities were extremely low, decreases with increasing depth in the core (Table 1). The mean value for the three Miocene

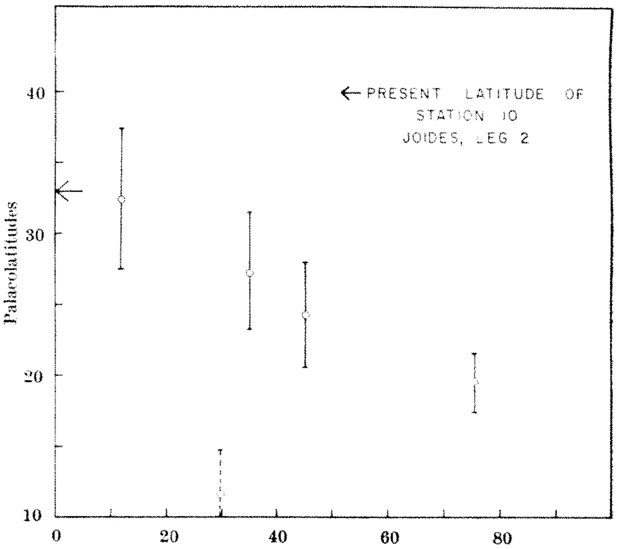
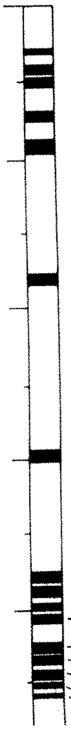


Fig. 1. Palaeolatitudes, with error bars, plotted against time.

Table 1. PALAEOMAGNETIC DATA FROM CORE 10, LEG II, JOIDES DEEP SEA DRILLING PROJECT (BASED ON TABLE 1 AND FIG. 10 OF OPDYKE[6])

Depth (m)	Core section	Core No.	Epoch	Age (m.y.)	Number of values	Mean intensity of remanence (10^{-6} e.m.u./g)	Mean absolute value inclination (degrees)	Standard error (degrees)	Palaeolatitude (degrees)
0			Pliocene						
		1 2 3	Miocene	12	14	3·90	51·6*	5·4	32·2
		5		30	9	1·88	22·3†	5·0	11·5†
			Oligocene						
100		7			10	3·98	45·7	5·0	27·1
			Upper Eocene						
				40					
		9	Middle Eocene	45	6	2·26	42·0‡	4·77	24·2
200									
			Lower Eocene						
300		10	U. Maastrichtian	65					
			L. Maastrichtian						
		11 12							
400		13 14	U. Campanian						
		16 17 18 19 20	Middle-Lower Campanian	76	20	6·12	35·5	3·1	19·6

* All data included. If one result with an anomalously low inclination is excluded, the mean inclination becomes 54·7°.
† Many intensities are unusually small in this case; mean inclination may be unreliable.
‡ Excluding two values with unusually low intensities ($< 10^{-7}$ e.m.u./g). If these are included, mean inclination becomes 34·2°.

cores is significantly greater than the mean for the Upper Cretaceous (Campanian). When plotted against age, the mean palaeolatitudes decrease uniformly from a latitude close to the 32·9° N latitude of station 10 to a latitude of 19·6° N (Fig. 1).

The Cretaceous palaeomagnetic pole for North America is well located at 69° N, 186° E on the basis of palaeomagnetic results from rocks on the continent[7]. The angular distance between station 10 and this pole is 71°. Station 10 is west of the mid-Atlantic Ridge on a part of the Atlantic sea floor which, according to the concept of plate tectonics, has remained rigidly connected to the continent of North America since the Cretaceous. The predicted inclination for the Cretaceous field at station 10 is 36° whereas the observed value for the Campanian cores is 35·5°. Though this evidence does not prove that the North American plate comprising the north-western Atlantic Ocean and the American continent has remained rigid for the past 80 million years, it is consistent with this assumption.

The internal consistency of the present results is encouraging in view of the many processes which may distort the magnetization of sediments during or after deposition[8]. Especially promising is the possibility of obtaining palaeomagnetic data from parts of the sea floor such as the South Pacific which, unlike the Atlantic, comprise plates that are not rigidly attached to continents. A comparison of palaeolatitudes based on data from unoriented cores collected at well separated sites would test the rigidity of large plates. More important, if it is possible in the future to collect cores that are oriented with respect to azimuth, complete polar paths may be determined for the oceanic plates.

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Deep Sea Tide Gauge with Optical Readout of Bourdon Tube Rotations

THE traditional method of measuring the tide by referring sea-level elevations to a stationary structure cannot be applied to the open ocean. Tidal pressure fluctuations on the sea floor, however, are a good indication of the mass variations of the water column. In order to detect changes of water head of 0·5 cm in 5 km depth an instrumental stability and resolution of 10^{-6} is required. Records obtained with vibrating wire transducers, corrected for temperature effects, indicate that this is possible[1-4]. The association of an adequately heat treated Ni Span *C* Bourdon tube with a sensitive optical detector[5] permits deep-sea tide gauges of great simplicity to be made, with ample resolution, negligible temperature dependency and predictable creep characteristics.

The instrument is contained within a heavy-wall aluminium case. The upper end of a freely hanging 42 turn Bourdon helix communicates with the outside. Attached to the lower end of the helix, a small finger supporting a mirror and a magnet dips into a cup of oil that damps its natural oscillations. The rotations generated by the changes of water head (5×10^{-6} radian per centimetre) are sensed by a frictionless, grid-type optical detector and a feedback system.

The functional diagram is shown in Fig. 1. At intervals controlled by a clock, a miniature lamp illuminates through a condensing lens the lower half of a ruling located in the focal plane of an objective in front of the mirror. The light focused on the mirror is reflected back through the objective to form an image of the lower half ruling on the upper half. As the mirror rotates, the ruling image moves with respect to the real ruling, modulating the transmitted light. Solar cells in a balanced configuration intercept it, thus converting the rotations into electrical signals.

Because the periodicity of the ruling is very small, the passage from maximum to minimum transmission requires only minute rotations, and so minute pressure changes. Because diffraction lessens the sharpness of the image, the required linearization is achieved as follows. The amplified signal is applied to a coil surrounding the magnet and the resulting magnetic field produces a torque that twists the Bourdon tube. Rotation of the mirror in turn modifies the signal until equilibrium is reached. The gain of the amplifier is large, and so the error signal is negligible and the meter that measures the nulling current, therefore, also reads the pressure changes.

Because of the periodicity of the rulings, there is a multiplicity of nulls. As the light is interrupted between readings, the system balances with respect to the closest one. The coil current therefore varies between limits $\pm I_{\max}$ and resets to $-I_{\max}+\Delta I$, instead of increasing to $I_{\max}+\Delta I$. So with proper adjustment of the sensitivity the recorded trace shifts to the left when about to run off-scale to the right, and vice versa. The size of the discontinuities generated in the record depends only on stable parameters—namely the grid periodicity, the focal length of the objective, and the elastic constant of the Bourdon tube. They constitute precise and permanent calibrations. The number of nulls depends on the excess width of the incident ruling with respect to the other. At present the grid periodicity is 0·0125 cm, the calibration constant 200 cm, and the available number of nulls approximately 100.

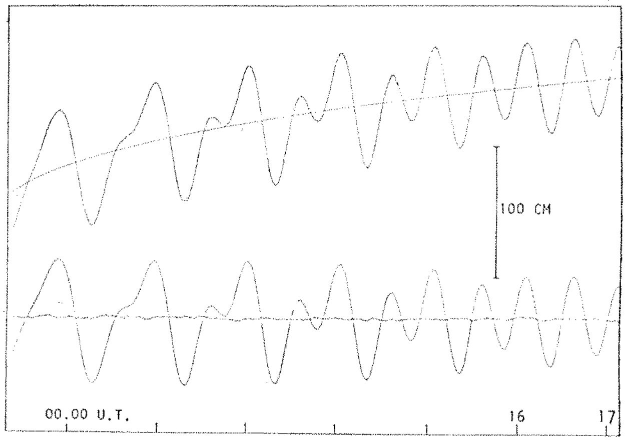

Fig. 2. The upper wavy line, slowly drifting upward, is a plot of the original data. The creep contribution is superimposed on it. The pure tidal and the noise contributions are plotted below.

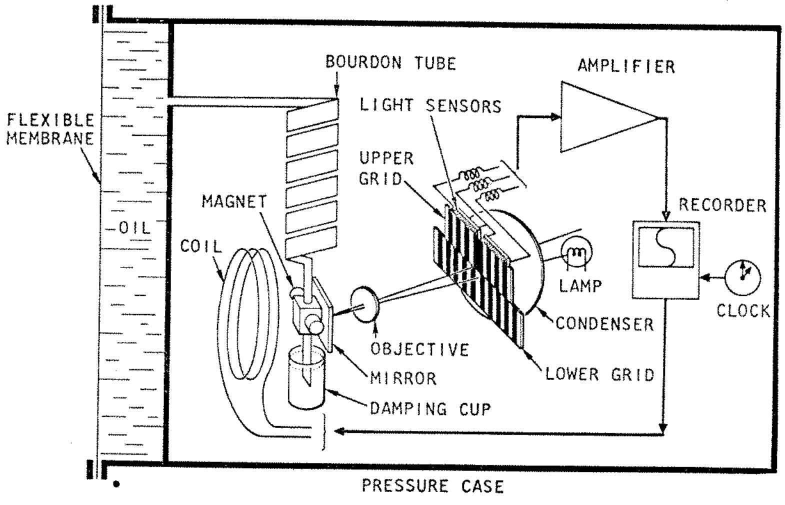

Fig. 1. Schematic diagram of the instrument.

Table 1. TRANSFER FUNCTIONS DESCRIBING TIDAL RESPONSE TO GRAVITATIONAL FORCES AND THEIR UNCERTAINTY

Constituents	Ang. Sp. (deg/h)	Ampl. (cm)	Observations Tsf. Fct. Mod.	Phase (deg)	La Jolla Ampl. (cm)	La Jolla Phase (deg)	Bogdanov Ampl. (cm)	Bogdanov Phase (deg)
*K*1	15·04107	26·3	2·44 ± 0·15	221 ± 5	32·8	207	36·9	221
*P*1	14·95893	8·5	2·39 ± 0·15	220 ± 5	10·6	192	11·1	220
*O*1	13·94304	14·3	1·87 ± 0·23	201 ± 10	21·0	183	25·6	210
*Q*1	13·39866	2·5	1·71 ± 0·28	187 ± 16	3·8	204	4·8	204
*M*2	28·98410	22·4	1·11 ± 0·09	115 ± 8	48·9	143	34·7	172
*S*2	30·00000	10·4	1·10 ± 0·11	97 ± 10	19·9	136	13·0	160
*K*2	30·08214	2·8	1·10 ± 0·12	96 ± 11	5·6	131	4·5	159
*N*2	28·43973	4·5	1·14 ± 0·10	124 ± 10	12·1	119	7·4	178

The inferred constituent amplitudes as well as the observed tidal constants at La Jolla and the prediction by Bogdanov at the point of observation are also indicated. The phase is referred to high equilibrium tide at Greenwich.

The instrument is designed for any oceanic depth. Precentring of the mirror to ± 400 m is required while a final centring using a simple optical sensor and a servomotor is activated after settling on the bottom. The instrument is fastened to a low-profile tripod and anchored in a manner similar to that used for deep-sea mooring of ocean floor magnetometers[6].

Twenty D-size batteries allow 28,800 data points per test, or 1 week of operation at 30 s sampling intervals, 10 weeks at 5 min intervals, and 1 year at 0·5 hour intervals.

A 7 day record obtained during October 1968, in water 4·4 km deep, 1,250 km off the coast of Baja California (position 24° 46·9′ N, 129° 1·1′ W) is shown in Fig. 2. The instrumental drift that was shown to obey Andrade's beta creep law[7,8] is removed easily and on legitimate ground. The tidal constituents can be estimated with an accuracy considerably better than that obtained with coastal records of similar length because of the extreme smoothness of the record and in spite of its short duration. The transfer functions between gravitational driving and tidal response have been estimated together with their uncertainties by a least squares analysis[9]. These estimates as well as the inferred constituent amplitude and phases are listed in Table 1. Comparison with the adjacent coastal amplitudes (Scripps Institution of Oceanography, La Jolla, California) indicates that the offshore attenuation of the semidiurnal tide is considerably larger than that of the diurnal tide. The observed amplitudes are consistently lower than those predicted by Bogdanov's mathematical models[10].

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Estimation of Fibril Lengths in Chrysotile Asbestos Fibres

CHRYSOTILE asbestos, the major fibrous serpentine polymorph, occurs naturally as veins in massive serpentine. It has been established[1,2] that chrysotile has a cylindrical crystal habit and occurs as fibrils 200 Å–400 Å in diameter. The orientation of the fibrils in the rock is such that the fibril axis is approximately perpendicular to the vein wall, forming a cross-fibre deposit, and the fibre length is frequently equal to the width of the rock vein; often the fibres grow from both walls and join at a central fissure or break. In commercial usage fibre length is an important characteristic, so various methods of length classification have been developed and applied to commercial raw material. These methods generally apply only to macrofibre bundles, however, each of which contains a large number of parallel fibrils. No data have been published for the fibril lengths of chrysotile, although it is apparent from examination of electron micrographs of dispersed chrysotile fibrils that they exceed several tens of microns in length. This agrees with an aspect ratio of 5,000 derived from strength measurements by Wronski[3]. In addition, Aveston[4] suggests that the somewhat broader fibrillar units of the amphibole asbestos crocidolite have lengths in excess of 1 mm. It is still uncertain, however, whether the lengths of individual chrysotile fibrils correspond to the length of the macro fibres or are shorter, because of defects introduced during growth or by subsequent geological faulting. This uncertainty limits the understanding and full utilization of the properties of chrysotile, in particular its mechanical properties.

With the increasing technological importance of chrysotile, particularly as a reinforcement in high strength materials, where aspect ratio is a critical parameter, it was necessary to undertake a detailed investigation of fibril length using a number of techniques, fuller accounts of which will be published elsewhere.

Samples for fibril length measurement were prepared by gentle stirring of fibre bundles in an aqueous surfactant solution producing dispersed, mainly single fibrils. Great care was taken to minimize fibre damage.

It is impossible to measure directly the lengths of long fibrils from electron micrographs such as Fig. 1, because their length greatly exceeds the field of view of the microscope at the magnification necessary to resolve individual fibrils. A statistical method has therefore been developed which relates the fibril length to the ratio of the number of fibrils to fibril ends visible in a field of view of known dimensions.

A feature of this method is that only individual fibrils are counted and therefore the length values relate to fibril units and not to macro fibres. The method has been verified using simple model systems of randomly distributed rods.

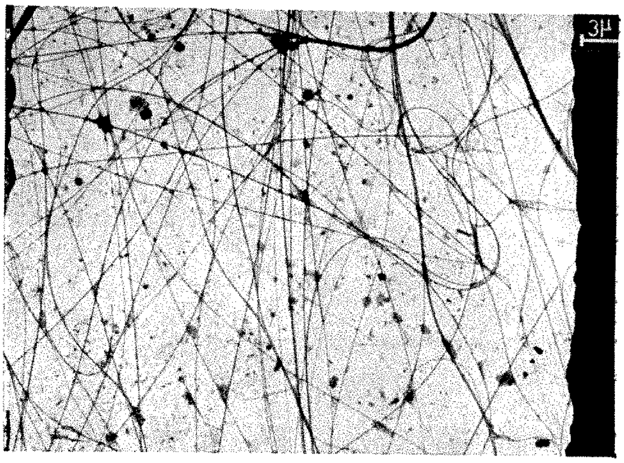

Fig. 1. Typical electron micrograph of long chrysotile fibrils.

Typical values of fibril lengths, estimated for some chrysotile samples, are shown in Table 1.

Table 1. COMPARISON OF ESTIMATED VALUES OF FIBRIL LENGTHS

Chrysotile sample	Mean estimated lengths (mm) Direct measurement from electron micrographs	Statistical measurement from electron micrographs	Drag reduction method	Viscosity method
Mechanically shortened fibril suspension for drag calibration	$1\cdot6\times10^{-2}$	—	Calibration sample	$1\cdot4\times10^{-2}$
Canadian (Cassiar)	$>1\times10^{-2}$	1·8	1·7	5–11
Canadian (Bells)	$>1\times10^{-1}$	1·4	1·7	2–2·5
South African (Kaapsche Hoop)	$>1\times10^{-1}$	4·0	3·9	—

Confirmation of the length values estimated by electron microscopy has been provided by examination of the rheological properties of aqueous suspensions of chrysotile. It has been suggested[5] that a relationship exists between the aspect ratio of fibres added to a turbulent flow system and the resultant reduction in frictional drag or increased rate of flow. An apparatus has been constructed and calibrated to measure this effect, from which values of aspect ratios of chrysotile fibrils have been estimated (Table 1) and lengths calculated, on the assumption of an average diameter of 400 Å.

In addition, attempts have been made to estimate the lengths of fibrils by viscosity measurements on aqueous suspensions. Lengths calculated from Burgers equations[6] have been found to disagree with directly observable lengths of mechanically shortened chrysotile fibrils in suspension. It has been shown[7], however, that curved fibrils deviate significantly from the Burgers model and can produce anomalously high values of aspect ratio, and it is therefore suggested that the curved, flexible nature of chrysotile fibrils (see Fig. 1) is responsible for this discrepancy.

From the work outlined here the following conclusions can be drawn. Carefully dispersed samples of chrysotile fibres, several centimetres in length, have been shown to be composed of fibrils at least several millimetres long—that is, within one order of magnitude of the macro-fibre length. This corresponds to an aspect ratio of approximately 10^5, an extremely high value for an inorganic single crystal whisker. There is, however, some evidence that even the gentle process of separating fibrils from their parent bundles could have resulted in some fibril shortening. The possibility therefore remains that the fibrils are of the order of centimetres long, and extend the whole length of the parent fibre bundles.

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Melting of Very Small Particles during Evaporation at Constant Temperature

THE dependence of the melting point of small solid particles on their size is implicit in the work of J. J. Thomson[1] on the effect of size on phase equilibria. It has been shown that large depressions of melting point are found when the diameters of the particles are about 20 nm. Takagi[2] and Blackman and Curzon[3] used electron diffraction techniques to study the melting of such small particles. The particle sizes in the latter case were determined by electron microscopy. Further systematic studies have been carried out by Wronski[4] with tin, and by C. J. Coombes (private communication) on lead (see also refs. 5–7).

A new method for observing the melting point of individual gold particles is described in this communication. Particles of solid gold were maintained at a constant temperature, somewhat below the bulk melting point, in a JEM 6A electron microscope. At this temperature, the rate of change in the size of the particles, caused by their evaporation, can be conveniently measured (see ref. 8 for similar measurements on silver). Instead of the monotonic evaporation curves of the kind found for silver particles, the results showed a distinct change in the rate of evaporation of particles (see Fig. 1) at a size which was dependent on temperature.

The change in evaporation rate is caused by melting of the gold particles. This change occurs because the solid gold particles have an evaporation coefficient less than unity[9], while the molten particles have an evaporation coefficient of unity.

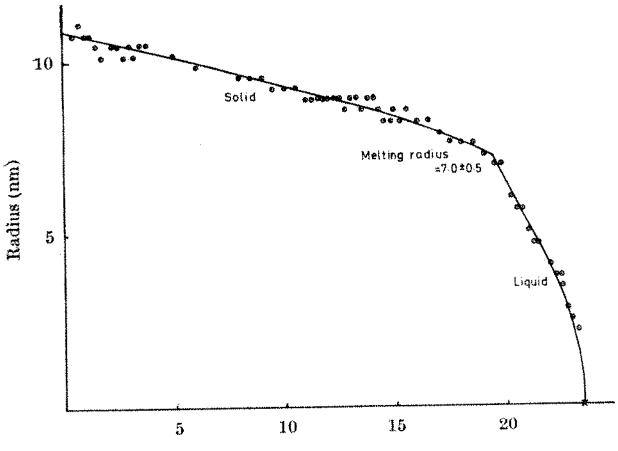

Fig. 1. The evaporation of a gold particle, $T = 1{,}229 \pm 5$ K. X marks the time when the particle became unresolved in the electron microscope image.

A series of experiments were carried out at different temperatures (T) over the range 1,150 to 1,330 K. These temperatures were determined from the rate of evaporation of the liquid particles; the radius of a particle at the melting point was taken at the sudden change in the rate of the evaporation curves.

It is to be expected that the reduced temperature, T/T_m (T_m being the bulk melting temperature), plotted as a function of the radius would give a universal curve for materials with similar properties and similar crystal structure. The results of Coombes (private communication), for lead, fit within experimental error the curve drawn for gold; surprisingly, the data for tin found by Wronski[4] also fit the same curve.

A detailed account of this work will be published later. One of us (J. R. S.) thanks the Science Research Council for a maintenance grant.

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Increasing Permeability of Polymer Membranes by Irradiation in the Fully Swollen State

THE permeability of polyolefin film may be made selective towards a particular solvent by annealing the film below its dissolution temperature in that solvent. For example[1,2], a selectivity towards *p*-xylene in a mixture of *o*, *m* and *p*-xylenes has been conferred on a polyolefin membrane in this way. Michaels and co-workers[3] have extended this to other solvents and were able to increase permeability as much as fifteen-fold relative to untreated membranes, but with reduced selectivity toward the permeants. These enhanced permeabilities were, however, not permanent. We demonstrate here[4] that when solvent annealing of a polyethylene film is followed by cobalt-60 γ-irradiation —while the film is still in contact with the annealing solvent—permeabilities are greatly increased. The enhancement produced by a combination of irradiation and annealing, however, is not only greater than that caused by annealing alone, but is far more permanent.

Polyethylene films were pretreated by soaking for about 48 h in solvent and then irradiated for different times in the same solvent. After irradiation, the films were dried before being placed in the permeation medium, except when this medium was identical with the irradiation solvent.

All permeation measurements and annealing of films were carried out at 30° C. During irradiation, the temperature of the medium fluctuated between 33° C and 38° C; the higher temperatures resulted from γ-ray heating but were not large enough to account for the observed increases in permeability. The experimental procedure and apparatus have been described[5]. The technique eliminated non-uniformity along the transport path within the films and yielded transport rates for films fully and uniformly equilibrated with the liquid permeation medium.

Table 1. POLYETHYLENE PERMEABILITY DATA AT 30° C

Run No.	Identification	$P\times10^7$ (cm²/s)	Remarks
1	TOC	6·28	
2	TOC	6·17	
28	TC(3)C	7·73	Not dried between irradiation and permeation
15	TOT	4·14	Preswollen in toluene, not irradiated
20	TOT	4·87	Preswollen in cyclohexane and dried before immersion in toluene for permeation studies
25	TOT	4·81	Run 25 is re-run of film from run 20 after it was soaked in toluene for almost 450 h
21	TC(1)T	4·79	
27	TC(1)T	5·31	Re-run of film from 21 after solvent annealing 550 h in toluene
22	TC(3)T	5·80	
26	TC(3)T	5·16	Re-run of film from 22 after solvent annealing 500 h in toluene
23	TC(8)T	7·00	
24	TC(24)T	7·91	
32	TC(24)T	7·61	Re-run of film from 24 after solvent annealing 520 h in toluene
15	TOT	4·14	
29	TT(3)T	4·96	Not dried between irradiation and permeation
30	TA(3)T	4·37	Solvent annealing in cyclohexane and dried before irradiation in air
31	TA(3)T	4·10	Irradiation dry in air
T1	BOT	5·45	All these films were dried for 6 h between irradiation treatment and permeability measurement
T5	BOB	4·56	
T3	TB(3)T	6·42	
T2	BB(3)T	6·71	
T6	BB(3)B	5·40	
T7	BT(3)B	6·71	
T4	BT(3)T	8·08	

Low-density polyethylene films were exposed to γ-radiation while fully swollen. The results are identified by the code $X_1X_2(t)X_3$. Here X_1 identifies the permeant or tracer molecule, X_2, the irradiation medium, and X_3 the permeation medium. The number in parentheses (t) is the number of hours the film was irradiated while it was in solvent X_2. C = Cyclohexane, T = toluene, B = benzene, A = air; 0 in position X_2 means that the film received no irradiation treatment.

The results are given in Table 1 and also plotted in Fig. 1 where the effects of irradiation in solvent on increasing permeability are evident. It is also possible to estimate the increase in selectivity with respect to benzene and toluene, if the film is irradiated in toluene. It is best to write the results as follows:

System	$P\times10^7$ (cm²/s)	Selectivity $= P_B/P_T$
BOT	5·45	1·31
TOT	4·14	
BT(3)T	8·08	1·63
TT(3)T	4·96	

Clearly, for the separation of small amounts of benzene from toluene, pre-irradiation of a toluene-annealed film for 3 h increases the selectivity by about 24 per cent. Pre-irradiation of the film in benzene and longer irradiation periods might cause even larger increases in selectivity.

Films prepared as we describe exhibit no substantial loss of enhanced permeability after treatment by annealing and irradiation and subsequent removal from contact with the solvent; the permeability has been rechecked for up to 500 h after such treatment and no loss of the enhancement was observed. The effect therefore lasts at least five times longer than that observed by Michaels[1,2].

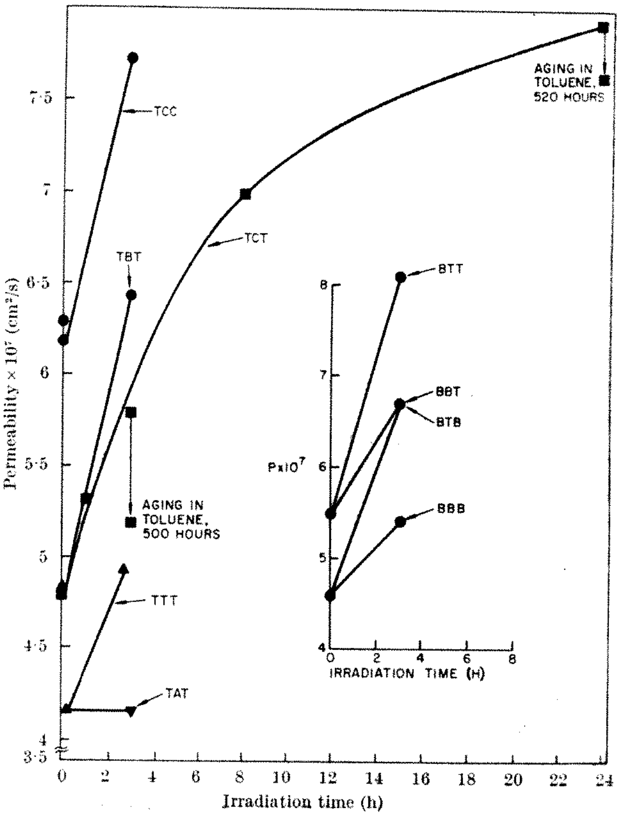

Fig. 1. The permeability data of Table 1 plotted to show the effect of irradiation time on membrane permeability. Dose rate = $0·85\times10^6$ rad/h; permeation temperature, 30° C.

In the light of Michaels's recent report[3], far greater enhancement of permeability might be expected if simultaneous annealing and irradiation are carried out at higher temperatures (but below the temperature of complete dissolution). Our experiments indicate that permeabilities were in some cases about doubled by annealing and irradiation at room temperature. Michaels *et al.*[3] reported permeability enhancement (of the non-permanent kind) up to fourteen times by solvent annealing at higher temperatures. Thus even greater permeability enhancement (which should also be permanent) would be expected by combined solvent annealing and irradiation of polymeric membranes at elevated temperatures.

To explain the process of solvent annealing, a polyolefin film may be viewed as a system of spherulitic polymeric crystallites embedded within an amorphous polymeric matrix. Diffusive transport within this system takes place predominantly within the amorphous part. When this system is annealed in a good solvent at an elevated temperature, the larger spherulitic crystallites may be expected to grow at the expense of the smaller ones, which simultaneously disappear. Thus the annealing process leads to larger crystals and a coarser network of spherulitic polymeric crystals within the amorphous polymeric matrix. The structure becomes more open and diffusive transport through this matrix of coarser, relatively impermeable spherulites is thereby enhanced.

The effect of γ-irradiation on the solvent-annealed polymer is presumably to produce free radicals at sites both within crystallites and within the amorphous matrix. This results in cross-linking which binds together the polymer chains within the enlarged crystallites into permanent structures that will not redissolve. Cross-linking of this type probably puckers the structure and destroys to some extent the crystalline regularity and perfection (cross-linking polymers by γ-irradiation is known to decrease crystallinity). Nevertheless, the net result would be to leave the former crystallites as dense, tightly bound entities. Moreover, interaction between free radicals produced within the amorphous phase and those produced at the surfaces of crystallites would bind polymer segments from the amorphous phase to the crystallites; this would produce additional holes within the permeable amorphous phase and thereby further enhance the permeability of the overall system.

Irradiation of swollen membranes seems to enhance their capacity to discriminate between molecules, as well as their permeability, to a degree associated with fully swollen polymers. The potential application of such membranes is evident in areas such as dialysis, ion-exchange, reverse osmosis and other separation processes.

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Non-dissociative Reaction of Oxygen with Metals

DURING our matrix isolation studies of the infrared spectra and structures of molecular species in metal oxide vapours[1-4], we have made some observations that are of interest in a completely different context—the primary reaction step in metallic corrosion.

Gaseous metal oxides may be formed readily as high temperature vapour species, either by heating the solid oxide or by passing oxygen gas at low pressures over the heated metal. Using both these methods we have obtained infrared spectra assignable to molecules M_xO_2 (M = Ge, Sn, Ga, In, Tl). Mass spectrometric evidence[5] suggests that M_xO_2 molecules may be important components of the vapours in numerous systems. Evidence that our species contain two equivalent oxygen atoms is based, in part, on the effect of ^{18}O-enrichment of the parent oxides, which then produce three isotopic molecules on vaporization: $M_x{}^{16}O^{16}O$, $M_x{}^{16}O^{18}O$, $M_x{}^{18}O^{18}O$.

When mixtures of $^{16}O_2/^{18}O_2$ gas, containing negligible amounts of the molecule $^{16}O^{18}O$, are passed over heated germanium, tin, gallium or indium, the spectra produced are identical with those obtained from the ^{18}O-enriched solid oxides, and prominent infrared bands are observed for the three isotopic species. Isotope scrambling therefore takes place in the formation of the oxide molecule. When the same gaseous mixture is passed over heated thallium, however, virtually no $Tl_x{}^{16}O^{18}O$ is produced, although $Tl_x{}^{16}O^{16}O$ and $Tl_x{}^{18}O^{18}O$ are readily identified (Fig. 1*b*). When the experiment is repeated with partially equilibrated oxygen gas, containing significant amounts of $^{16}O^{18}O$, the corresponding mixed oxide is observed (Fig. 1*c*). These spectra may be contrasted with that obtained from vaporization of solid thallium oxide, where complete randomization occurs (Fig. 1*a*).

We are therefore led to the inescapable conclusion that, in the formation of the oxide molecule Tl_xO_2 by direct oxidation of the metal, both the oxygen atoms must be derived from the same molecule of oxygen. Chemisorption of oxygen on metals is almost invariably dissociative, so that oxygen isotopes are randomized in the formation of volatile polymeric oxide species. On thallium, the initial chemisorption step seems to be non-dissociative.

Dissociative adsorption of any diatomic reactant (O_2, S_2, and so on) implies a sufficiently high energy of formation of the initial adsorption complex. Ehrlich[6] has suggested that, if the adsorption energy is great enough, one atom may be trapped at the site of primary adsorption while part of the adsorption energy is transferred, as translational energy, to the second atom. This then migrates several unit spacings across the surface before it is bound. Isotope scrambling is a necessary consequence of such a process. There is too little information about the adsorption energy of oxygen on metals to warrant any wide comparison between the elements, but it may be surmised that there must be a critical value, below which the act of chemisorption is non-dissociative. In the absence of such data, the enthalpy of formation of the oxides might be taken as a crude measure of the metal–oxygen interaction energy within any one group. For group IIIB, the ΔH_f° values (kilocalories/g atom oxygen) are

$$Ga_2O,\ -82;\ 1/3\ In_2O_3,\ -74;\ Tl_2O,\ -42;$$
$$1/3\ Ga_2O_3,\ -86$$

On this basis it might be predicted from the thermochemical data that few other metals would show the same behaviour as thallium. It may be relevant, however, that Michell and Smith[7] have shown that the oxidation of cadmium in air forms CdO_2, not CdO. Here, too, the chemisorption step involves electron transfer to molecular oxygen, without dissociation. In most cases this may be a very transient precursor stage; in a few cases the reaction products reveal it.

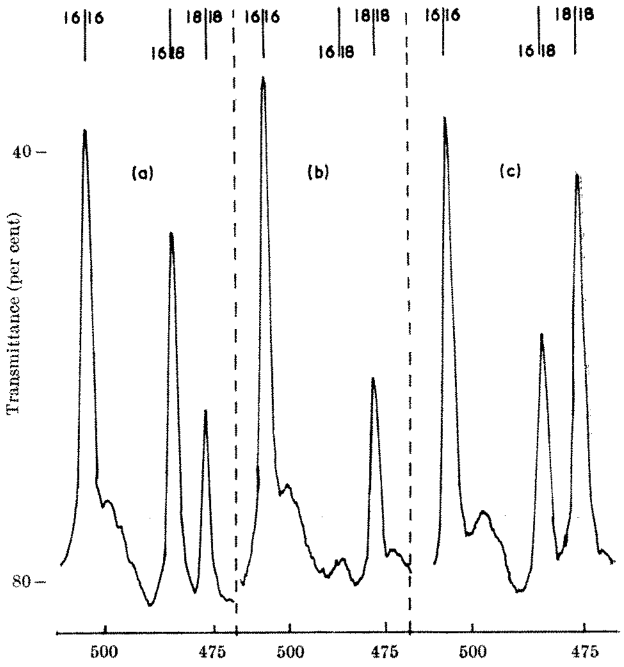

Fig. 1. Infrared spectra (cm⁻¹) of thallium oxide species obtained from (*a*) solid Tl_2O labelled with ^{18}O; (*b*) $^{16}O_2/^{18}O_2$ mixture over heated Tl metal; (*c*) $^{16}O_2/^{16}O^{18}O/^{18}O_2$ mixture over heated Tl metal.

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Saturation of a Molecular Absorption Line at 890 GHz

It has been shown[1,2] that the molecule 1 : 1 difluoroethylene CF_2CH_2 has an absorption line close to the main 890 GHz emission line of the HCN laser. The results suggest that the line is about 0·5 MHz from the laser line centre and has an absorption coefficient of $\alpha = 0{\cdot}0008 \pm 0{\cdot}0003$ nepers cm^{-1} at 0·1 torr. Experiments carried out in the laser cavity[1] provided some evidence for saturation of this line. We describe here more explicit observations of saturation both intra and extra cavity.

Two HCN lasers were used in these experiments. A hemispherical configuration, 4 m long with an axial mode diameter of 2·5 cm at the plane mirror and 10 cm diameter at the curved mirror, was used for the intra-cavity measurements. The 1 m absorption cell was placed at the plane mirror to maximize the laser power density in the absorbing difluoroethylene. The laser output was coupled out of the cavity by a beam splitter and detected either by a Golay cell or by a silicon crystal–tungsten whisker diode. The cavity modes were swept by a linear mirror drive or by an oscillating loudspeaker coil on which the plane mirror was mounted. The output power of the laser was of the order of 10–20 mW and the power inside the cavity was approximately 400 mW with no absorbing gas.

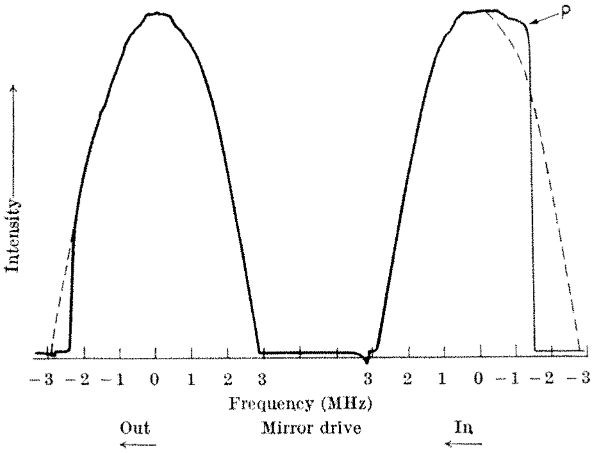

Fig. 1. Laser interferogram with CF_2CH_2 in the cavity. 890 GHz fringes.

With 0·05–0·100 torr of CF_2CH_2 in the cell, Q-switching effects caused by bleaching of the absorber were observed. Fig. 1 shows a typical cavity resonance curve using the linear motor mirror drive. The dotted lines are the symmetrical curves obtained with no gas in the cell. It will be noted that the form of the resonance depends on the direction of the mirror drive: switching on and off occurs at different levels of laser output as would be expected for a saturation phenomenon. Fig. 1 also shows a small Q-switch gain over the CW power level at P. This was the maximum gain observed and there was no evidence of repetitive Q-switching when the plane mirror was oscillating over the resonance at approximately 1 kHz frequency. The switching time was found to be of the order of 100 μs. The absence of any large Q-switch gain confirms the observations of Jones *et al.*[3], who used a rotating mirror in a Q-switched CW HCN laser. The apparent gain could not be interpreted as an inverse Lamb dip effect caused by saturation hole burning in difluoroethylene gas[4,5]. At 0·05 torr the collision broadened line width is approximately 1·0 MHz[1], which is considerably less than the laser tuning width (8 MHz) and hence distinctive enough to show up independently of the mirror drive direction. In any case, in this configuration of laser and absorption cell the power would homogeneously broaden the dip at the low pressures necessary for $\Delta\nu_{\text{collision}}$ to be less than $\Delta\nu_{\text{Doppler}}$ (see below).

The external cavity absorption experiments were carried out in a 6 m long brass cell 2 cm in diameter. The HCN laser used was near confocal, 6·5 m long, 10 cm in diameter and had a power output of about 50 mW coupled out of the cavity by means of a 2·5 cm hole in one of the mirrors. The absorption coefficient as a function of laser power for 0·04 torr gas pressure is shown in Fig. 2. The broadening caused by saturation is immediately obvious. Calculations from these data are complicated by the nature of the line. Thus the Doppler width is approximately 1·4 MHz and the collision self-broadening width for 0·04 torr is 0·8 MHz[1]. A convolution of the two shapes for the line[6] leads to a width of 2·0 MHz which is observed, although the uncertainty in pressure measurement is ± 20 per cent.

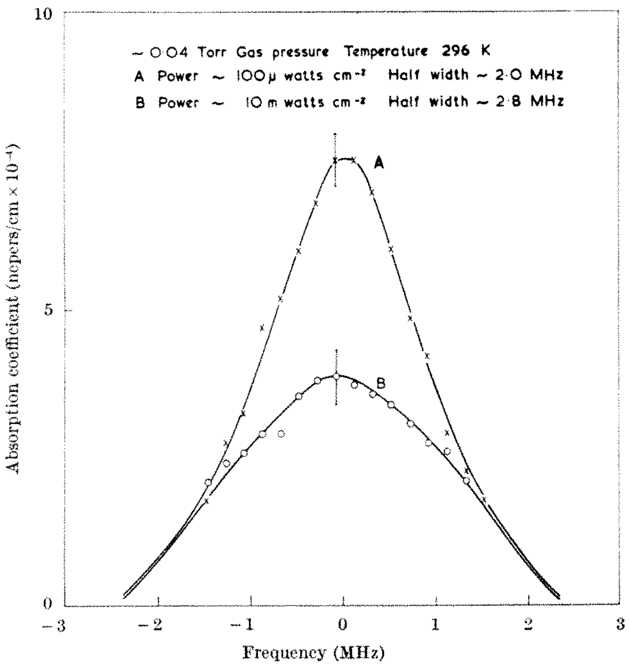

Fig. 2. Saturation in CF_2CH_2 at 890 GHz, 6 m external cell.

A general form for the absorption coefficient for saturation of a homogeneously broadened line is given by Townes and Schawlow[7] as follows:

$$\alpha = \alpha_0 \left[1 + \frac{32\pi|\mu_{01}|^2 P}{3ch^2\Delta\nu^2} \right]^{-1} \tag{1}$$

where P is the radiation power, $|\mu_{01}|^2$ the dipole matrix element for the transition and $\Delta\nu$ is the full line width. This is expected to be modified for inhomogeneous (Doppler) broadening[9], but it will serve for the purpose of discussion.

Approximate calculations indicate that the rotational transition in CF_2CH_2 is in the region (J, Ka, Kc) 49, 38, 11→48, 38, 10 (unpublished work of G. Duxbury and D. Finnigan) which is approximately 750 cm^{-1} above the ground level. Absolute assignment is complicated by the lack of centrifugal distortion information. Using the measured dipole moment[7,8] of 1·385 Debye leads to a value of $|\mu_{01}|^2$ of 0·5 to 1 Debye[2]. Substituting in the above equation gives $\alpha = \alpha_0/2$ for P equal to about 10 mW cm^{-2}. This value has only qualitative significance because the power as measured by a Golay cell may be uncertain.

An experiment has been carried out in which the output of the laser was split into two beams which were directed through the absorption cell in opposite directions. Preliminary results indicate that a saturation dip is observable in difluoroethylene in these conditions. This may be of use in the frequency stabilization of the HCN laser.

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BIOLOGICAL SCIENCES

Blocking of the Lymphocyte Antigen Receptor Site with Anti-immunoglobulin Sera *in vitro*

ALTHOUGH the events occurring during the induction of an antibody response are not clearly understood, it is evident that antigen must react with some type of receptor which is either free in serum or present at a cell surface. Various experiments[1-3] in which labelled antigen is allowed to react *in vitro* with lymphocytes suggest that a very small proportion of all lymphocytes does in fact have the ability to recognize and bind a given antigen. The specificity of this binding reaction between antigen and lymphocyte was demonstrated in both adoptive transfer experiments[4] and *in vivo*[3]. Electron microscopic studies indicate that the reactive lymphocytes possess localized receptor sites for antigen on their surface[5]. Preliminary studies[2] showed that pretreatment of these cells with a polyvalent anti-immunoglobulin serum inhibited antigen binding and suggested that the antigen receptor site involves some type of immunoglobulin molecule. We now report in more detail on the nature of the receptor sites on lymphocytes and suggest that interaction of antigen with these sites is crucial in the induction of the antibody response.

Normal CBA mouse spleen or peritoneal cell suspensions were preincubated with a 20–50 per cent final concentration of different, specific anti-mouse immunoglobulin sera (or control normal sera) for 2 h in an ice bath, and the cells washed by centrifugation through a foetal calf serum gradient. The cells were then allowed to react with ^{125}I or ^{131}I-labelled antigens at 0° C for a further 30 min, centrifuged through serum gradients and finally smeared on glass slides for the preparation of autoradiographs. The procedures for labelling of antigens, preparation of cell suspensions, autoradiography and counting of labelled cells have been described[2]. Antigens used[2] were flagellin and polymerized flagellin from different *Salmonella* strains and haemocyanin (*Jasus lalandii*).

In a second type of experiment, cell suspensions pretreated as above with anti-globulin sera, were injected into lethally (850 rad) irradiated syngeneic mice (groups of eight per cell suspension) together with an immunogenic dose of unlabelled antigen. The recipient mice were then bled either 8 or 14 days later and serum samples titrated for antibody activity.

Anti-mouse immunoglobulin sera were prepared by repeated immunizations of rabbits with purified mouse myeloma proteins emulsified in Freund's complete adjuvant. The sera were rendered heavy chain specific by appropriate absorptions with either mouse immunoglobulin light chains or myeloma proteins of another immunoglobulin class. The specificity of the absorbed antisera was verified by use of the very sensitive inhibition of precipitation assays of ^{125}I-labelled mouse myeloma proteins as previously described[6].

Table 1. EFFECT OF ANTI-IMMUNOGLOBULIN SERA ON THE ABILITY OF NORMAL MOUSE SPLEEN CELLS TO BIND ^{131}I-LABELLED ANTIGENS

Rabbit antiserum pretreatment (0° C, 2 h)	Percentage of control antigen uptake*	Mean per cent control†
Normal serum	69, 74, 110, 138	98
Anti-human serum albumin	101, 125	113
Polyvalent anti-LH chain	<10, 11, 12, 23	<14
Anti-L-chain	6, 10, 11, 14, 33, 38, 90	29
Anti-L-chain preabsorbed with mouse L chains	100, 170	135
Anti-α-heavy chain	71, 90, 105	89
Anti-γ_1-heavy chain	90, 94, 100	95
Anti-γ_2-heavy chain	72, 99, 150	107
Anti-μ-heavy chain	7, 33, 51	30

* Each value represents an individual experiment performed with either *Salmonella* flagellin or haemocyanin as antigen. Each value is the percentage of antigen uptake for the serum treated cells as compared with the control cells with no serum. Antigen uptake is scored as the number of labelled lymphocytes per 10^6 cells.

† Mean of the individual values.

The results with all antisera are presented in Table 1, in which the percentage of control antigen uptake is shown. All controls (no serum pretreatment) showed the usual level of labelled cells. Each value represents an individual experiment, and the mean values clearly indicate that significant inhibition of antigen uptake by cells was achieved only with antisera containing antibodies either to immunoglobulin light chains or to μ (macroglobulin) heavy chains.

To determine whether these same cells with IgM surface receptors were indeed involved in the primary antibody response, antiserum treated cell suspensions were injected together with various antigens into irradiated mice. The results (Table 2) indicate that marked suppression of antibody formation has been achieved by pretreatment of cells with either anti-light chain serum or an antiserum containing predominantly anti-μ-chain antibodies. This particular anti-μ-chain serum was unabsorbed and contained traces of anti-light-chain activity. In view of the concentration of the anti-light-chain serum required to achieve inhibition, it is unlikely that the trace of anti-light chain antibody present in the anti-μ-chain serum was responsible for its inhibitory activity. Specific anti-γ-chain sera did not inhibit this response.

A preliminary examination has also been made of the nature of antigen binding receptors on lymphocyte-like cells from other sources. Peritoneal cell suspensions (from the normal peritoneum and not oil induced exudates of adult mice) contain a very high proportion of cells which bind antigen. With both antigens used inhibition of binding occurred after treatment of cells with a polyvalent anti-immunoglobulin serum (24 per cent of control uptake) although this reduction was not as marked as with adult spleen cells. A similar degree of inhibition (29 per cent) was observed with newborn mouse spleen cells and a greater inhibition (7 per cent) with spleen

Table 2. EFFECT OF ANTI-IMMUNOGLOBULIN PRETREATMENT OF NORMAL MOUSE SPLEEN CELLS ON THEIR ABILITY ADOPTIVELY TO TRANSFER PRIMARY ANTIBODY PRODUCTION TO IRRADIATED RECIPIENTS

Antigen	Serum preatment (0° C, 3 h)	Antibody titre in irradiated recipients (log$_2$)*		Significance (P value) compared with normal serum or no serum
		Day 7–9	Day 14	
Polymerized flagellin SW 1338	No serum	2·5	—	
	Preimmune normal serum	2·5	—	
	Anti-μ (trace L)	1·2	—	< 5, > 1%
Polymerized flagellin SL 871	Normal serum	7·3	—	
	Preimmune normal serum	8·2	—	
	Anti-L-chain	3·7	—	1%
Brucella abortus	Preimmune (anti-L) normal	—	8·5	
	Anti-L-chain	—	4·1	1%
	Anti-μ-chain (trace L)	—	5·0	1%
Sheep erythrocytes	Preimmune (anti-L) normal	—	3·5	
	Anti-L-chain	—	1·0	1%
	Anti-μ-chain (trace L)	—	< 1·0	1%

In each experiment the preimmune normal serum is the preimmunization bleed of the anti-μ or anti-L rabbit.

* Bacterial immobilization was used for flagellin, agglutination assay for *Brucella* and sheep erythrocytes.

cells from 3 day old mice. Further studies are in progress to determine whether the incomplete inhibition observed with newborn mouse spleen and adult peritoneal cells is attributable to a phagocytic type of cell not involved in specific antigen recognition.

Our results, combined with related studies[2,3,5], strongly suggest that lymphocytes from unimmunized mice have at their surface IgM macroglobulins which specifically bind antigens. Other studies have indicated that the normal lymphoid cell population contains two discrete populations of cells: thymic derived cells involved primarily in cellular immunity and bursal (or bursal equivalent) derived cells involved in humoral antibody formation[7]. From the result of the *in vivo* cell transfers in these and other studies[3], it is probable that these lymphocyte-like cells are involved in humoral immune responses. Thus differentiation of haemopoietic stem cells towards antibody producing precursor cells would involve the expression of the light and μ-chain immunoglobulin genes, with a resulting localization of the IgM molecules on the cell surface. This is supported by the observation[8] that IgM is the first immunoglobulin synthesized within the bursa of Fabricius itself, which is the first location of immunoglobulin synthesis in chickens.

This suggests that further differentiation toward cells secreting large amounts of antibody is induced by contact of antigen with the surface IgM receptor. Further, if all normal antibody producing precursor cells carry these IgM receptors, indicative of μ-chain gene activity, then the resulting IgG antibody formation which occurs in many responses may reflect an intracellular switch from the μ-chain gene to the γ-chain gene, as has been suggested[9]. Alternatively, different cells may possess IgM, IgG and other immunoglobulin class receptors, but there is a preponderance of cells with IgM receptors.

These results may also be compared with those from a similar approach, involving the use of the same antisera to identify the nature of the antigen recognition site on cells mediating cellular immunity[10]. Cell suspensions pretreated with antisera were assayed for immunological competence in either graft versus host reactions or in the cellular transfer of delayed hypersensitivity. In the latter systems, almost complete suppression of activity was achieved with the anti-light-chain serum, but by contrast with the results now reported, no anti-heavy-chain antibody (including anti-μ-chain antibody) had any inhibitory activity.

Thus the initial contact between antigen and normal unprimed cells involved in either cellular or humoral immunity involves a cell surface bound immunoglobulin molecule as the unit of recognition, and it is intriguing to consider that a different class of immunoglobulin is involved in the two systems. Further studies with ^{125}I-labelled purified anti-immunoglobulin antibodies, now in progress, may demonstrate directly the location of the immunoglobulin receptors on the cell surface.

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In vitro Reactivity of Lymphocytes to Particulate and Soluble Antigens

NUMEROUS reports have documented the response of sensitized peritoneal exudate cells to various antigens, including PPD, BCG and ovalbumin[1-8]. By contrast, migration inhibition studies of sensitized peripheral lymphocytes without the addition of macrophages have either been inconsistent or negative[9,10]. Johanovsky, using a splenic explant system, obtained consistent migration inhibition to a number of particulate and soluble antigens; his system did, however, contain a number of different cell types[11]. The previous reports by Bendixen and Søborg that the migration of sensitized human leucocytes could be inhibited by various particulate antigens such as *Brucella* or organ extracts suggested that the sensitized lymphocyte could, in fact, respond to an antigenic stimulus provided the antigen was in a certain form[12-14]. The concept of a particular antigenic configuration being required for the stimulation of the sensitized lymphocyte was further confirmed by our consistent observation of the inhibition of migration of sensitized lymphocytes to transplantation antigens, streptococcal structures, and heterologous erythrocytes (refs. 15, 16 and our unpublished work). In each case the presence of a particulate form of the antigen seemed to be indispensable for inhibition of migration.

To assess further the difference between particulate and soluble antigens with respect to the inhibition of migration of sensitized lymphocytes, tuberculin sensitized human and rat lymphocytes were exposed to various tubercle products. The response to particulate and soluble antigens was assessed by (1) the inhibition of migration of lymphocytes from capillary tubes, and (2) increased DNA synthesis in response to antigenic confrontation. The animals were derived from our own breeding colony and consisted of an inbred strain of Fischer rats, maintained by strict brother to sister mating: they were fully inbred. The rats were sensitized to tuberculin by an intraperitoneal and bilateral subcutaneous injection of a total of 1 ml. of Freund's complete adjuvant (FCA) (Difco Laboratories). Ten days after the first injection a second injection of an equal volume was given. The animals were used in these experiments 8 to 12 days after the second sensitization.

In the experiments with human lymphocytes, normal healthy individuals, as well as patients from the local hospital with diseases other than tuberculosis, were used. All of these patients gave a history of having previously been sensitized to tuberculin by vaccination with BCG. The time since vaccination ranged from 5 to 50 yr. Previous exposure to the tubercle bacillus was ascertained by intradermal skin testing with first and intermediate strengths PPD (Difco Laboratories). The reactions were graded as positive if an induration of more than 5 mm was observed 48 h after intracutaneous injection of the material.

The separation and preparation of lymphocytes from rats and humans for the inhibition of migration studies and the ^{14}C-thymidine incorporation experiments were as previously described[15–17]. In general, the lymphocyte preparations contained less than 10 per cent granulocyte contamination in these studies. For the migration studies, the percentage of migration was calculated as follows:

$$\frac{\text{Area of migration with antigen}}{\text{Area of migration without antigen}} \times 100$$

DNA synthesis induced by the different antigens was determined by adding 0·1 μCi ^{14}C-thymidine (Radiochemical Centre, Amersham) for 24 h to each tube. Preliminary experiments indicated that the optimal time for addition of the isotope was 96 h after the initiation of the experiments. When phytohaemagglutinin (PHA) was used, the ^{14}C-thymidine was added 48 h after initiation of culture. The tubercle bacilli used in these experiments were cultured by the State Bacteriological Institute, Stockholm. These bacilli were harvested and killed by heating. One to two million organisms per ml. were used for most experiments. Purified protein derivative (PPD) was supplied by the State Serum Institute, Copenhagen, without any preservative and contained 1 mg per ml. of PPD. This solution was diluted to the appropriate concentration with the previously described medium.

Table 1 illustrates the results obtained with sensitized human and rat lymphocytes to the tubercle bacillus and PPD. At concentrations of 1×10^6–2×10^6 organisms per ml., tubercle bacilli significantly inhibited the migration of sensitized lymphocytes, whereas there was no response to 20 or 50 μg of PPD per ml. Sensitized rat spleen cells responded similarly to the tubercle bacillus while again no response was observed with the soluble PPD. Control non-sensitized animals did not respond significantly to either form of the antigen. Similarly, skin test negative human subjects failed to show a significant inhibition.

Table 2 illustrates the response of tuberculin sensitized human lymphocytes to the tubercle bacillus in the capillary migration system and to PPD as it affects the level of DNA synthesis after five days of culture with the antigen. Clearly the lymphocytes of tuberculin sensitized human subjects do respond to soluble PPD antigen as measured by the incorporation of ^{14}C-thymidine, but the correlation between the two tests—namely, inhibition of lymphocyte migration and the level of DNA synthesis—was variable. Because the latter method measures the response of both antigen-sensitive cells and antibody-producing cells after prolonged incubation, variations between the two tests might be expected.

The use of a pure lymphocyte population in the capillary migration system has been the subject of considerable dispute. While both positive (ref. 12 and our unpublished work) and negative[10] results have been reported, successful inhibition of lymphocyte migration may well depend on a particular antigen configuration. Here a significant response was observed with sensitized lymphocytes in either human or rat subjects if the antigen was in a particulate form. By contrast, negligible inhibition of migration was observed when soluble PPD was present in the medium. A significant response was observed, however, when sensitized lymphocytes were exposed to the soluble antigen during a five day period of culture. We suggest that in order for lymphocyte migration inhibition to occur, the antigen must be in a particulate form and perhaps of a certain configuration. The basis of this reaction and the reason why a particular form of antigen seems to be essential for inhibition of lymphocyte migration are being studied.

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Table 1. THE EFFECT OF TUBERCLE BACILLUS (TB) AND PPD ON THE MIGRATION OF HUMAN LYMPHOCYTES AND RAT SPLEEN CELLS

Antigen tested	TB (1×10^6/ml.)		TB (2×10^6/ml.)		PPD (0·02 mg/ml.)		PPD (0·05 mg/ml.)	
	No.	Inhibition*	No.	Inhibition*	No.	Inhibition*	No.	Inhibition*
Sensitized human cases	15	26·7 (±2·0)	8	32·4 (±3·8)	10	4·0 (±1·3)	5	0·6 (±0·6)
Non-sensitized human cases†	11	4·5 (±1·9)	—	—	—	—	—	—
Sensitized rats	11	28·9 (±3·4)	4	27·0 (±3·9)	4	4·4 (±2)	7	4·0 (±1·6)
Non-sensitized rats	8	4·9 (±1·9)	—	—	6	6·6 (±1·8)	—	—

* Mean percentage inhibition (± *S.E.*).

† Tuberculin test: PPD at intermediate strength (0·001 mg) was negative.

Table 2. A COMPARISON OF THE EFFECT OF TUBERCLE BACILLUS (TB) ON THE MIGRATION OF SENSITIZED HUMAN LYMPHOCYTES TO THE EFFECT OF PPD ON DNA SYNTHESIS

Cases	Tuberculin test*		Per cent inhibition of migration with TB		^{14}C-thymidine incorporation (c.p.m.)		
	First strength PPD (0·0001 mg)	Intermediate PPD (0·001 mg)	1×10^6/ml.	2×10^6/ml.	Normal medium	PHA (1/100)	PPD (0·02 mg/ml.)
D.M.	10	—	30	—	20 (12–31)	1,531 (1,441–1,709)	549 (291–762)
R.R.	11	—	25	—	84 (65–104)	1,410 (963–1,397)	1,444 (1,084–1,865)
L.L.	8	15	15	23	141 (102–182)	1,671 (1,542–1,785)	737 (525–865)
B.H.	15	—	37	37	75 (59–93)	1,820 (1,643–1,996)	1,010 (891–1,104)
J.G.	12	—	27	—	104 (97–108)	2,304 (1,990–2,935)	497 (337–578)
G.T.	20	—	38	—	32 (26–44)	923 (849–1,057)	306 (295–328)
I.S.	12	—	24	—	34 (18–42)	1,488 (1,412–1,592)	218 (187–288)
H.U.	10	—	24	—	90 (61–119)	2,240 (1,863–2,693)	768 (600–991)
W.L.	10	—	29	49	123 (100–145)	1,052 (949–1,190)	643 (453–823)
L.A.	10	—	28	27	105 (71–129)	1,456 (1,228–1,508)	395 (333–450)
L.D.	10	—	24	23	31 (21–37)	1,713 (1,676–1,730)	1,221 (936–1,721)
B.S.	7	12	11	24	28 (15–47)	1,436 (1,300–1,611)	1,785 (1,543–1,848)
Mean + *S.E.*			26·0 ± 2·2	30·6 ± 3·6	72·2 ± 12	1,587·0 ± 117·7	797·8 ± 136·6

* Refers to mm of induration.

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Suppression and Enhancement of Antibody Formation by Alteration of Ca^{2+} Levels

IT is becoming clear that membrane events are important in antibody formation—in the recognition and handling of immunogens, in the activation and multiplication of antibody-forming cells, and in the interaction of the several cell types involved[1]. Various agents (for example, chlorpromazine, epinephrine, antilymphocyte sera and phytohaemagglutinin) known to alter membrane permeability can change the magnitude and, in some cases, the specificity of the antibody response, but most of the reported effects had been transient and weak[2-6]. Because calcium ions are known to influence many membrane-associated events[7-23], we tested the influence of altered levels of Ca^{2+} on antibody formation *in vivo*, and have observed striking changes which, depending on the timing of the alteration, involved either enhancement or suppression of responses to sheep red blood cells (SRBC) in mice.

Female CFW mice (6–8 weeks old) were immunized intravenously with 10^8 SRBC and the number of antibody-forming spleen cells (AFC) was determined 48 or 72 h later[24]. Treatments designed to change levels of Ca^{2+} in the circulation were started at different times before or after immunization. To elevate serum Ca^{2+} levels (by approximately 2 mequiv/l.), 400 µg of $CaCl_2$ (dihydrate, Merck) was injected intravenously, at 6 h intervals, a total of five times (each dose being approximately 1 mequiv/kg). To lower serum Ca^{2+}, porcine calcitonin (Armour, Lot K 423–079, obtained through Dr J. W. Bastian) was injected subcutaneously, 1 MRC unit per mouse, every 8 h, a total of three times. Groups of five mice were used.

Results were calculated in terms of multiples or fractions of average responses in experimental groups compared with the average response in untreated, immunized animals. In the case of the 48 h determinations, the elevation of Ca^{2+} levels, if initiated either before or 12 to 24 h after antigen administration ("early" or "late"), significantly inhibited responses; by contrast, if elevation of Ca^{2+} levels was started at the time of antigen administration, or within 6 h thereafter, the responses were stimulated. The effects of calcitonin were essentially the reverse of the effects obtained after Ca^{2+} elevation (in a subsequent test a 3·5-fold stimulation was obtained when calcitonin treatment was started after 18 h). Seventy-two hours after immunization, the enhancement of antibody formation resulting from Ca^{2+} elevation, at or shortly after the time of immunization, was even more striking (Fig. 1), but the suppressive effects, noted in assays 48 h after immunization, were no longer detectable. In fact, early or late modification of Ca^{2+} levels now showed some stimulatory effects.

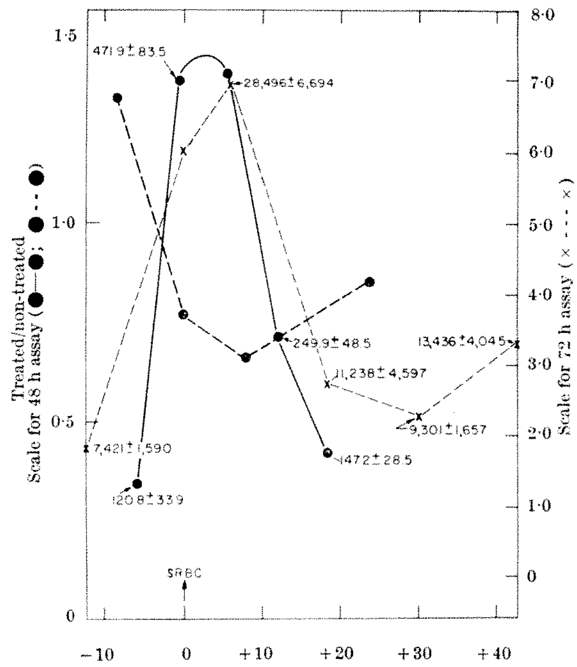

Fig. 1. The influence of $CaCl_2$ and calcitonin, administered at different times before and after immunization with SRBC, on the number of antibody forming spleen cells (AFC) in mice 48 h and 72 h after immunization. The results are given in terms of the ratio of average number of AFC in treated groups to the average number of AFC in the control group receiving SRBC only. Each group contained five CFW females; the figures adjacent to some of the points indicate average AFC/10^8 spleen cells ± *S.E.* ●——● and × - - - ×, $CaCl_2$ (400 µg/mouse intravenously every 6 h; five times). Control value for 48 h assay: 348·1 ± 12·0; for 72 h assay: 4,139 ± 912. ● — — — ●, Calcitonin (1 MRC unit/mouse subcutaneously every 8 h; three times).

The inhibitory effect of early (−6 h) Ca^{2+} elevation, detectable 48 h after immunization, was overcome by the administration of poly A·poly U, a known stimulator of antibody responses[25] if given at the time of antigen injection; poly A·poly U did not, however, increase the enhancement effect of Ca^{2+}.

The inhibitory effects of "early" and "late" Ca^{2+} elevation may be understood in terms of interference with the handling of the immunogen by macrophages on the one hand, with the interaction of lymphocytes with antigen, required for "driving" the antibody response[6], on the other. Similarly, the stimulatory effects of "early" and "late" calcitonin administration could be attributed to an increased permeability of macrophages and lymphocytes. What is difficult to explain, so far, is the stimulatory effect of Ca^{2+} elevation (and the reverse effect of Ca^{2+} depression), when the modification of Ca^{2+} levels is initiated at or shortly after the time of immunization. Perhaps the handling of an excessive and interfering amount of antigen is "shut off"; or perhaps the cells involved in the early events leading to antibody formation act more effectively. In view of the several cell types and many events involved in antibody formation, however, a conclusive interpretation of the modifying effects of elevated Ca^{2+} levels cannot be given.

Calcium ions influence several types of membrane-associated events, which may be involved in modifying immune responses. Ca^{2+} is important in regulating the deformability and viscosity of the membrane[7]; the plasma membrane becomes extremely rigid when the intracellular Ca^{2+} is raised[8]. Such events may affect endocytosis, which seems to play a part in antibody formation. Low extracellular Ca^{2+} increases the permeability of the cell membrane to various ions[9,10] and the resulting changes in cytoplasmic electrolyte levels may affect the activity of intracellular enzymes involved in antibody formation. Cytoplasmic Ca^{2+} levels have been recognized as the primary link in the general concept of stimulus-response coupling which embraces all the events between stimulation of the cell membrane (for example, by antigen) to the responses of the cell[11-14]. For example, histamine release from mast cells during the antigen–antibody reaction requires the presence of Ca^{2+} in the medium[15,16]. Ca^{2+} is also important in controlling the electrical excitability and membrane potential of cell membranes[17-19]; it influences complement-dependent reactions[20], and modifies membrane-associated enzymes, such as Mg-ATPase[21,22]. Finally, contacts among various cells are required for the activation of immune responses and Ca^{2+} is known to be important for the maintenance of cell junctions and communicability[23].

Further studies of the ways in which changes in Ca^{2+} levels may affect antibody formation by modifying membrane-associated events may aid significantly in the identification of critical membrane events in immune responses, and even permit a better manipulation—both in a positive and negative direction—of antibody formation. It may even be possible, through appropriate modifications of Ca^{2+} levels, to achieve some control over the direction of immune responses in terms of humoral antibody formation versus cell-mediated immunity.

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Secretory Piece linked to IgM in Individuals deficient in IgA

THE IgA in exocrine secretions, by contrast with that in serum, is an 11S dimer containing an additional antigenic fragment, variously referred to as the transport piece, secretory piece or simply as the "piece" (ref. 1). Piece determinants, as detected by double diffusion precipitation in agar with a specific antiserum, occur in small amounts in the sera of up to 40 per cent of individuals with untreated adult coeliac disease, and in 9 per cent of healthy persons[2]. During a search for piece determinants I have examined the sera of thirty-four patients deficient in IgA. Thirty had hypogammaglobulinaemia or dysgammaglobulinaemia and had an increased susceptibility to infections. Four had other symptoms, but no history of repeated infections.

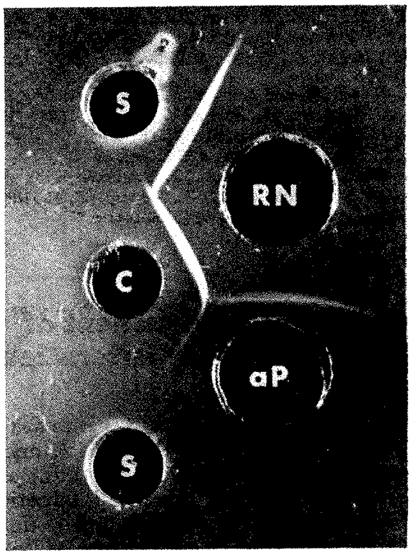

Fig. 1. Reaction with isolated colostral IgA (*C*) of the piece specific antiserum (*aP*), absorbed with normal human serum (*S*). A reaction of identity is given with the antibodies to piece determinants in an anti-secretory IgA antiserum (*RN*), provided by Dr R. W. Newcomb of Denver, Colorado.

A specific anti-secretory piece antiserum was prepared by injecting a preparation of human salivary IgA into a rabbit and absorbing the antiserum with whole human serum, with non-IgA-containing preparations of human salivary amylase and with an α_2 protein found in saliva[2]. Fig. 1 demonstrates the specificity of this antiserum. Three patients' sera gave a positive reaction with anti-piece antiserum, although two showed no evidence of alpha chain determinants in their sera and the third only a trace. The individuals suffered from recurrent infections —Table 1 lists their serum immunoglobulin levels. To detect small amounts of piece or alpha chain determinants, a sensitive haemagglutination-inhibition technique was developed. Colostral IgA, prepared by a slight modification of the method of Newcomb *et al.*[3], was coated on sheep erythrocytes using glutaraldehyde[4]. Using three minimum agglutinating doses of the anti-piece antiserum, exocrine IgA could be detected at a concentration of 0·4 μg/ml., and with a similar dose of an anti-alpha chain antiserum, monomer serum IgA could be detected at a

Table 1. IMMUNOGLOBULIN LEVELS (mg/ml.) IN THREE IgA DEFICIENT SERA

Serum	IgG	IgM	IgA	IgD
C. G.	2·5	2·24	< 0·03	< 0·01
G. K.	9·4	1·02	< 0·03	< 0·01
M. C.	2·3	0·15	< 0·03	< 0·01
Normal	6·8–23·5	0·32–2·4	0·81–6·3	< 0·01–2·2

Results were obtained by a single radial diffusion method.

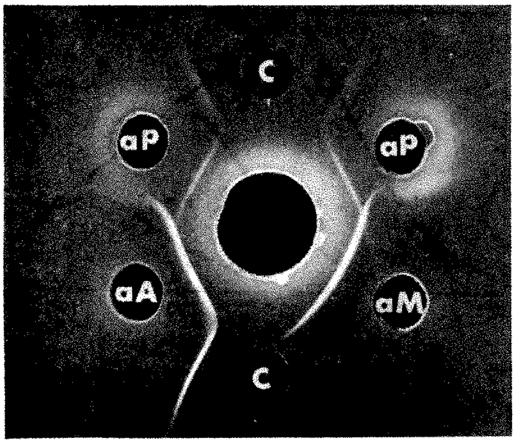

Fig. 2. Antigenic analysis of the pooled concentrated fractions of the first protein peak from gel filtration on 'Sephadex *G*-200' of the serum from a healthy individual (central well). *aP* = anti-piece antiserum; *aA* = anti-alpha chain antiserum; *aM* = anti-μ chain antiserum; *C* = isolated colostral IgA.

concentration of 0·2 μg/ml. The sera C. G. and G. K., at a dilution of more than 1 in 40, inhibited the agglutination of the colostral IgA coated cells by the anti-piece antiserum, but not that by an anti-alpha chain antiserum; the serum M. C. inhibited agglutination by both antisera to the same degree.

In the sera of individuals with adult coeliac disease and healthy individuals showing piece determinants, this activity is usually confined to the IgA region on electrophoresis, and to the fractions of the exclusion peak from gel filtration of serum on 'Sephadex *G*-200' (ref. 2). The haemagglutination-inhibiting activity has the same electrophoretic location and occurs in the retarded fractions of the first protein peak from the 'Sephadex' column, while the principal portion of the serum IgA was eluted in the second protein peak. Antigenic analysis of the first peak fractions containing this activity, from a healthy individual having an appreciable amount of piece determinants in the serum (Fig. 2), indicated that piece determinants in the fraction were linked to IgA, although a considerable portion of the IgA in the fraction was not so linked. On the other hand, there was a reaction of non-identity with the IgM-anti-μ chain line.

The sera of C. G. and M. C., deficient in IgA but containing piece determinants, were subjected to gel filtration on 'Sephadex *G*-200'. Piece determinant haemagglutination-inhibiting activity occurred only in the main fractions of the exclusion peak. Antigenic analysis of the pooled concentrated fractions of the exclusion peaks from the 'Sephadex *G*-200' gel filtration of these sera (Fig. 3) gave for each material a reaction of non-identity with IgA, and spurred with the IgM-anti-μ chain line, although clearly a considerable amount of IgM was present which did not contain piece determinants. Electrophoresis in agar of whole serum (G. K.) showed that the piece determinants had a similar distribution to the serum IgM.

Table 2. PIECE DETERMINANT HAEMAGGLUTINATION-INHIBITION TITRE AFTER PRECIPITATION OF SERUM IMMUNOGLOBULIN

		Immunoglobulin precipitated			
Sera		IgG	IgM	IgA	IgD*
IgA deficient	C. G.	45	2	—	50
	G. K.	60	2	—	50
Healthy	A.	80	55	4†	60
	E. S.	70	60	16†	90

* Anti-IgD added in antiserum excess.
† Anti-alpha chain antiserum added to the point of slight antigen excess.

Attempts were made to remove piece determinant haemagglutination-inhibiting activity by precipitation of the immunoglobulins from the IgA deficient sera, and from the sera of two healthy individuals, by specific anti-immunoglobulin heavy chain antisera, prepared in sheep by methods described by Rowe *et al.*[5] (Table 2). The activity was removed by precipitation of the IgM, but not of the IgG, in the IgA deficient sera; in the sera of the healthy individuals it was removed by precipitation of the IgA—but not of the IgM or IgG—or by the addition of anti-delta chain antiserum in antibody excess.

Some individuals with a deficiency of IgA have an excess of IgM in their secretions[6-8], although no evidence of linkage of this IgM to secretory piece was obtained by Brandtzaeg *et al.*[8]. Exocrine secretions from our three patients were not available, but the duodenal juice from another patient deficient in IgA was studied. This patient had no history of recurrent infections, and the juice showed no alpha chain determinants, but contained 7 mg/100 ml. of IgM and a trace of IgG. During gel filtration of this juice on 'Sephadex *G*-200', piece determinants were located by haemagglutination-inhibition in the IgM containing fractions of the exclusion peak, and after the fractions of this peak were pooled and concentrated, these determinants were assigned to the IgM by antigenic analysis in agar as in the experiment of Fig. 3. Again the larger proportion of the IgM did not seem to be so linked.

The association of IgM with secretory piece in individuals lacking IgA raises a number of questions. It has been suggested[9] that, in normal circumstances, secretory piece forms a primary non-covalent bond with exocrine IgA which is stabilized by the formation of disulphide bridges. The nature of the bond with IgM is unknown, but it could be formed similarly. The specimens we studied were too small to allow an elucidation of this problem and the amount of IgM linked to secretory piece represented

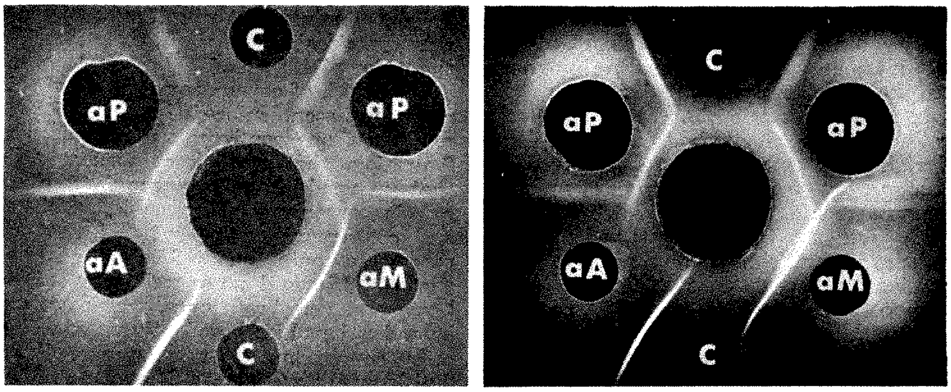

Fig. 3. Antigenic analysis of the pooled concentrated first peak fractions from the gel filtrations on 'Sephadex *G*-200' of two IgA deficient sera, M. C. (left) and C. G. (right). The pooled fractions are in the central well in each case, and the reagents around the peripheral wells are arranged as in Fig. 2.

only a small fraction of the IgM in each. Using more sensitive methods, it may well prove that the amount of secretory-piece-linked IgM is always small; it may, indeed, be dependent on the proportion of a sub-class of IgM in the secretions. The function of this additional determinant is unknown, but it may increase the resistance of exocrine IgA to digestion by proteolytic enzymes, and it may also do this for IgM.

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Natural Selection versus Gene Uniqueness

SMITH[1] has compared protein evolution with a popular word game. Just as the object of the game is to pass from one word to another by changing one letter at a time, so in the process of evolution one protein is changed to another by changing only one gene nucleotide at a time. As in the word game each change must result in an English word, so in protein evolution each nucleotide change must result in a viable protein.

I would point out, however, that it is not sufficient for each nucleotide change to result in only a viable protein—a better protein must be produced, such that natural selection would tend to favour it over the old protein.

Smith points out that, for evolution by natural selection to occur, functional proteins must form a continuous network which can be traversed by unit mutational steps without passing through non-functional intermediates. As a matter of fact, the protein network must have even a more stringent property, namely the possibility of traversing the network by unit mutational steps always proceeding to a more adaptive protein. Unless a mutation leads to a phenotype of greater selective value, a transition from the old phenotype would simply not be made. Thus when Smith asserts that his parameter f must be greater than 10^{-3}, he should be talking not about the fraction of viable proteins that can be derived by unit mutational steps, but about the fraction of proteins that represent improvements.

Although Smith gives the impression that he is disagreeing with Salisbury[2], and that a value of 10^{-3} for f is small, close examination reveals that the points Smith raises actually support Salisbury's contention. The value of f suggested by Smith means that an enormous number of unit mutational improvements are possible. If this is the case, the gene is far from unique. This was Salisbury's contention: the concept of evolution by natural selection and the concept of the uniqueness of the gene are incompatible.

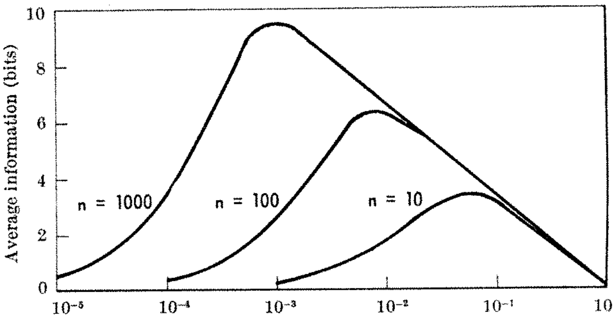

Fig. 1. Average information that can be transmitted into a species in 10^{12} to 10^{14} trials or fewer. The average information is plotted as a function of the fraction of sequences that represent better adapted phenotypes.

I have previously described this situation in terms of the amount of information that can be incorporated into the genetic message by the process of random mutation and natural selection[3]. The increasing adaptivity of a species of organism to its environment through an evolutionary process was viewed as a process of transmitting information from the environment into the species. The advantage of considering the problem as one of information transmission is that, in computing the expected amount of information incorporated into the genetic message, one simultaneously takes into account the effect of the probability of achieving an improvement, which is a monotonic increasing function of f, and the information added, which is a monotonic decreasing function of f.

Fig. 1 shows the average amount of information that can be transmitted into the genetic message in one evolutionary step. The average information is plotted as a function of f for various values of n, the number of nucleotides available for change. An evolutionary step is here defined as a sufficiently large number of trials (that is, births), perhaps as many as 10^{12} to 10^{14}. If an entire gene is available for change, then the $n = 1{,}000$ curve shows that a maximum amount of information of about 9·5 bits can be gained by the gene if $f \approx 10^{-3}$. If, on the other hand, only small sections of the gene are available for change—such as the active site—then the maximum amount of information is correspondingly smaller.

In a previous report[3] I have indicated how the expected information depends on the amount of grammatical structure, if any, that must be evolved along with the necessary phenotypic improvements. Calculations there show that, when a requirement for a grammatical structure is included, the maximum average information that can be transmitted into the genetic message is considerably smaller than that shown in Fig. 1.

A natural question to ask then is: where did all the information come from that is now in the gene? As Salisbury points out, even if only 10 per cent of the DNA represents real information, this still means something of the order of 10^8 bits.

We could, of course, say that all of this DNA does not really represent information in the sense that it only represents one configuration out of many possible configurations that could have occurred. This would mean that we would have to argue for the non-uniqueness of existing organisms. How could we then explain parallel and convergent evolution? If the eye, for example, represents only one of about 10^{10^6} possible forms that could have done as well, why is it that the invertebrate and vertebrate eyes, which are supposed to have been developed independently, seem to be so much alike?

In conclusion, after examining Smith's arguments, Salisbury's contention still seems to stand: the concept of evolution by random mutation and natural selection and the concept of the uniqueness of the gene are apparently contradictory. Further, if we should take the view that the gene is not unique, then we have to begin revising our present ideas of the phylogenetic tree in order to remove the concepts of convergent and parallel evolution.

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Induction of DNA Polymerase in Mixed Lymphocyte Cultures

HISTOCOMPATIBILITY is frequently determined by serological typing of lymphocytes for HL–A alloantigens[1]. This method is restricted by the availability of specific antisera and may be hampered by undetected antigens[2]. There is, however, no restriction on the choice of antigenic determinants in mixed leucocyte culture reactions. When lymphocytes from the peripheral blood of two individuals are mixed and cultured together, the extent of stimulation seems to be a function of disparities in histocompatibility[4]. "One way stimulation" can also be studied by preventing replication of either set of lymphocytes by prior treatment with mitomycin C (ref. 5) or by X-irradiation[6]. The morphological observation of blastogenesis and measurements of the uptake of ^{3}H-thymidine into cellular DNA have been used to monitor stimulation, but they have deficiencies (see, for example, refs. 7, 8).

A more reliable parameter for quantitating the extent of DNA replication in mixed lymphocyte cultures may be to assay DNA polymerase activity, *in vitro*[10], in cultures containing lymphocytes from two unrelated individuals.

Lymphocytes were isolated from the blood of healthy volunteers by the method of Cooper[9]. The final preparation of lymphocytes was suspended at a concentration of $7\cdot5\times10^5$ cells per ml. in Eagle's MEM (Spinner modification) (GIBCO, Grand Island, NY), containing 20 per cent foetal calf serum, 1 per cent L-glutamine, penicillin (100 units per ml.) and streptomycin (100 μg per ml.). The cell suspensions were incubated at 37° C in 16 × 125 mm disposable plastic culture tubes. After incubation for the times indicated in Fig. 1, the cells were washed and centrifuged at 2,500*g* in 0·15 M KCl. The final cell pellet was disrupted by rapid freezing and thawing in 0·1 ml. of a solution made up of 20 per cent glycerol, 0·02 M potassium phosphate buffer (*p*H 7·4), 0·004 M reduced glutathione, and 0·001 M potassium EDTA. DNA polymerase activity was determined as previously described[10] using the entire broken cell suspension from each culture as an enzyme source.

The DNA polymerase activity of cultured human lymphocytes is shown in Fig. 1. Essentially no stimulation occurred when lymphocytes from only one individual were in culture (LA or JB, 1 ml, cultures). The slight increase in activity of JB lymphocytes might have resulted from an immunological reaction directed against a component added to the culture medium, for example penicillin, foetal calf serum or streptomycin[11]. When cells from both individuals were cultured together in the same tube (LA plus JB, 2 ml. cultures) however, the DNA polymerase activity was markedly stimulated to an extent greater than the sum of the two individual curves at any time after 36 h. The periodic fluctuation of DNA polymerase activity in the mixed cultures is significant and may reflect changes in the population dynamics of these partially synchronized cells. As previously shown, phytohaemagglutinin (PHA) added to lymphocyte cultures from a single individual results in an even greater increase of DNA polymerase activity (JB plus PHA)[10].

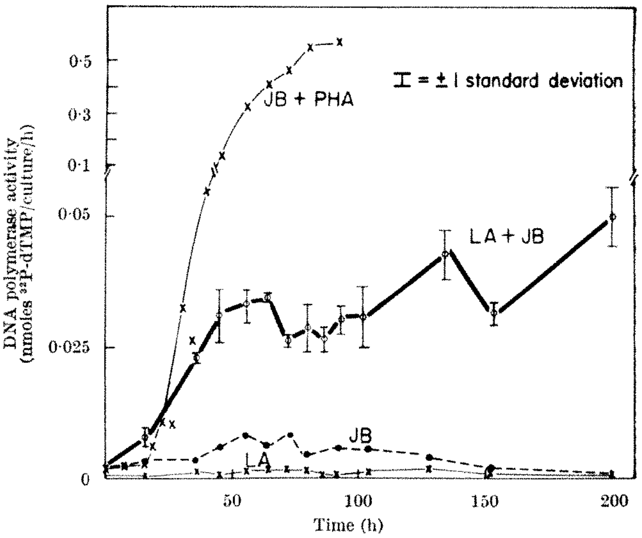

Fig. 1. Stimulation of DNA polymerase activity by lymphocyte interactions. All results were obtained from aliquots of lymphocytes from the two individuals (LA and JB) which were cultured simultaneously in triplicate in the presence and absence of PHA. Individual cultures contained 1 ml. suspensions of lymphocytes from LA or JB, while the mixed culture contained 1 ml. of cells from each of the individuals. In control experiments the activities of the cultures were proportional to the amount of cells. For example, 0·5, 1·0 and 2·0 ml. of a lymphocyte suspension which was cultured for 72 h had polymerase activity of 3, 5, and 8 pmoles, dTMP/culture/h; similar cultures incubated with (PHA) had a polymerase activity of 120, 248, and 500 pmoles, dTMP/culture/h, respectively. One ml. portions of lymphocytes from JB were incubated with 0·025 ml. of PHA–M (General Biochemicals, Ohio). DNA polymerase activity was measured as the incorporation of an appropriately labelled deoxynucleotide into an acid-insoluble product. The reaction mixture in a total volume of 0·3 ml. is the following: 25 μmoles Tris–maleate buffer, *p*H 8·0; 3 μmoles $MgCl_2$; 1 μmole KCl; 0·3 μmole β-mercaptoethanol; 25 nmoles each of dATP, dCTP, dGTP, and 10 nmoles of [α-^{32}P]-dTTP (about 5×10^4 d.p.m. per nmole) (International Chemical Nuclear, City of Industry, California); 255 nmoles of "activated" calf thymus DNA and 0·1 ml. of the final lymphocyte suspension. Incubation was for 1 h at 37° C and incorporation was determined after washing the acid-insoluble material by centrifugation[9]. Incorporation without incubation was 1·0 pmoles dTMP/culture.

In other studies we have presented evidence that as an index of lymphocyte stimulation, DNA polymerase is a reliable parameter for measuring the replicating response of lymphocytes to a mitogenic stimulus[12]. Because DNA polymerase activity is assayed *in vitro* it is not affected by concentration of metabolites in the cultures. The sensitivity of this assay is, for the most part, limited only by the specific activity of the labelled deoxynucleoside triphosphate precursors. With ^{32}P or ^{14}C-labelled deoxynucleoside triphosphates as precursors, DNA polymerase activity can be determined on the same cultures which previously incorporated ^{3}H-thymidine into cellular DNA. Assays of DNA polymerase activity and thymidine incorporations are not only supportative but are also useful in studying their interrelationships.

Phytohaemagglutinin, an extract of the red kidney bean, *Phaseolus vulgaris*, causes a similar transformation, though the extent of the response is much greater[13]. Whether or not the effect of phytohaemagglutinin is the same as that resulting from cell interactions in the mixed lymphocyte culture reaction is unknown. The increase in DNA polymerase activity in both phenomena could, however, result from an increased requirement for DNA polymerase activity associated with the transformation of well differentiated lymphocytes into actively replicating lymphoblasts. This transformation may be relevant for the expression of the immunologic properties of these cells.

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Molecular Weight and Chain Length of Rabbit Reticulocyte rRNA

THE molecular weight of mammalian ribosomal RNA has been the subject of various studies. Estimates of molecular weight of RNA by sedimentation and viscosity from Jensen sarcoma[1], liver[2] and HeLa cell[3] ribosomes are between 0·58 and 0·67 × 10^6 daltons for the small subunit and between 1·64 and 1·9 × 10^6 daltons for the large subunit. Estimates made by measuring the length of RNA molecules are 0·86 and 0·83 and 1·73 and 1·61 × 10^6 daltons for the small and large subunits of erythroblast and HeLa cell rRNA when compared with the length of *Escherichia coli* rRNA[4]. Estimates made by exonuclease digestion, when compared with *E. coli* RNA, give values of 0·4 and 0·8 × 10^6 daltons for the small and large subunits of HeLa cell rRNA[5]. I now present data which suggest that the RNA from rabbit reticulocytes consists of two main subunits of molecular weight 0·65 and 1·53 × 10^6 daltons and that in most conditions of isolation there is a small molecule of RNA associated in equimolar amounts with the large RNA subunit.

Reticulocyte polyribosomes were isolated in the absence of magnesium and the ribosomal subunits fractionated on sucrose gradients after treatment with EDTA[6]. Narrow cuts from the large and small ribosomal subunit peaks were taken to minimize cross-contamination. RNA was extracted from the ribosomal subunits using guanidinium chloride[7,8]. Polyacrylamide gel electrophoresis of the RNA revealed little cross-contamination between the large and small subunits. Periodate oxidation and condensation with isoniazid were performed as described previously[7]. Excess isoniazid was removed by chromatography on 'Sephadex *G*-75'.

Previous experiments have shown the usefulness of this method in determining molecular weights by yielding a value of 27·0 × 10^3 daltons for *E. coli* transfer RNA, in agreement with values of 24–27 × 10^3 daltons found by sequence analysis[7]. Midgley[8] has confirmed this finding and extended it to calculate the molecular weight of *E. coli* tRNA and 16S and 23S rRNA as 24 × 10^3 and 0·53 and 1·0 × 10^6 daltons, in good agreement with measurements by sedimentation and viscosity[9,10].

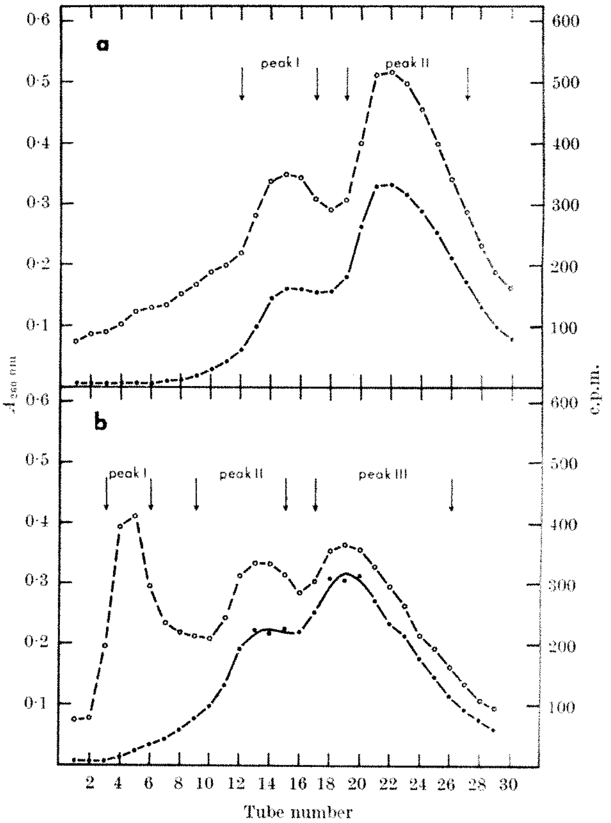

Fig. 1. Sucrose gradients of RNA fractions after periodate oxidation and condensation with isoniazid. All gradients were 17 ml. linear 5–20 per cent in sucrose in 0·1 M sodium acetate (*p*H 5·0). Centrifugation was at 25,000 r.p.m. at 4° C in a Spinco 'SW 25·3' swinging bucket head. Time of centrifugation was 20 h for (*a*) and 18 h for (*b*). (*a*) Untreated RNA from the large ribosomal subunit. (*b*) RNA from the large ribosomal subunit heated in 0·5 per cent sodium dodecyl sulphate in 0·1 M sodium acetate at 60° C for 10 min. 0·1 ml. of each fraction was diluted with 0·9 ml. of 0·1 M phosphate (*p*H 7·0) for measurement of absorbance at 260 nm; 0·5 ml. of these fractions was mixed with 4 ml. of a scintillation mixture containing 20 per cent Beckman 'BBS 3' solubilizer and 80 per cent of 0·5 per cent 2,5-diphenyloxazole and 0·5 per cent 1,4-*bis*-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene. The efficiency of counting tritium in this system was 19·1 per cent for (*a*) and 19·7 per cent for (*b*). Areas marked were collected by precipitation with 2 volumes 95 per cent ethanol, with the addition of 1·0 mg of carrier RNA for (*b*), I, for further analysis. ●—●, Absorbancy; ○ - - - ○, radioactivity.

The low values for the molecular weight of the rRNA isolated from ribosomal subunits compared with the values obtained from sucrose gradient fractionated total rRNA (Table 1) indicated that more than one molecule of RNA was present in the large subunit, most probably as a small molecule of RNA bound to the 28S RNA. To determine if this were the case, guanidinium chloride extracted RNA from the ribosomal subunits was heated in 0·5 per

Table 1. MOLECULAR WEIGHT OF RNA FROM 40S AND 60S RIBOSOMAL SUBUNITS TREATED IN DIFFERENT WAYS BEFORE AND AFTER SUCROSE GRADIENT SEDIMENTATION

Treatment	Molecular weights of RNA (daltons × 10^{-6}) Small subunit Before fraction-ation	Small subunit Main 18S peak after fraction-ation	Large subunit Before fraction-ation	Large subunit Main 28S peak after fraction-ation
None	0·432	0·688	0·742	1·18
0·5 per cent sodium dodecyl sulphate at 60° C before periodate oxidation and isoniazid reaction	0·368	0·650	0·755	1·53
7 M urea after isoniazid reaction	0·473	0·686	0·810	1·13
*p*H 3·0 after isoniazid reaction	0·475	(Not measured)	0·834	1·13
* Total ribosomes with guanidinium chloride followed by sucrose gradient fractionation	—	0·66	—	1·38
* Total ribosomes with 0·5 per cent sodium dodecyl sulphate at 37° C followed by sucrose gradient fractionation	—	0·79	—	1·48

* From ref. 14.

Molecular weights of RNA were calculated by estimating the number of grams of periodate oxidized RNA which reacted with one mole of isoniazid. RNA was estimated by its absorption at 260 nm using an extinction coefficient of 23 for a concentration of 1 mg/ml. in a 1 cm cell.

cent sodium dodecyl sulphate in 0·1 M sodium acetate (*p*H 5·0) at 60° C for 10 min and then oxidized and condensed with isoniazid. Other samples of guanidinium chloride isolated RNA from the ribosomal subunits were oxidized and condensed with isoniazid and then treated with 7 M urea in 0·1 M sodium acetate (*p*H 5·0), and 0·1 M sodium acetate–HCl (*p*H 3·0) for 15 min at 0° C. Sodium dodecyl sulphate releases a "6S" RNA from HeLa cell 28S rRNA[11]; 7 M urea destroys hydrogen bonding[4]; while titration of RNA to *p*H 3·0 readily breaks down hydrogen bonding in RNA and effectively reveals "hidden breaks" caused by nuclease action[12].

Fig. 1 shows sucrose gradient analyses of control and sodium dodecyl sulphate treated RNA coupled with ^{3}H-isoniazid. The profiles of the 7 M urea and *p*H 3·0 treated RNA were identical in all respects with the control. Table 1 estimates the molecular weight of the RNA fractions before and after sucrose gradient fractionation. The sodium dodecyl sulphate removed a small RNA of molecular weight less than 60,000 daltons from the 28S RNA isolated from the large ribosomal subunit. Neither the *p*H 3·0 nor the 7 M urea treatment released this RNA and the RNA after these treatments had essentially the same estimated molecular weight as the control. The change in the molecular weight of the 18S RNA from the small subunit on sucrose gradient fractionation is independent of the treatment of the RNA and may reflect contamination by a small amount of tRNA. That the release of a "6S" molecule from the 28S RNA is specific and not the result of degradation can be demonstrated by end group analysis after enzymic digestion. Fig. 2 illustrates radio paper electrophoretograms of pancreatic ribonuclease and ribonuclease T_1 digests of 28S RNA isolated from sucrose gradients of control and sodium dodecyl sulphate treated RNA. It is clear that while the control 28S RNA has two different end groups with sequences -pGpU and -GpCpU (unpublished results), the sodium dodecyl sulphate treated 28S RNA has the sequence -pGpU and the "6S" RNA has the sequence -pGpCpU. No difference was detected between the 18S RNA end groups from any of the treatments; one sequence, -pUpA, is found.

Because the chain length determinations agree very well with the best estimates of molecular weight by physical methods I conclude that the reticulocyte 18S and 28S ribosomal RNAs are single chain molecules with molecular weights of $0·64 \times 10^{6}$ and $1·5 \times 10^{6}$ daltons respectively. The lengths measured by Granboulan and Scherrer[4] may be explained by assuming that the treatment with 7 M urea does not break down the hydrogen bonded helical loops and that there is a different percentage of loops in the two subunits[13].

It is very difficult to reconcile my results with those of Riley[5]. The presence of a small molecule attached to the 28S RNA would cause an alteration in the initial slope of his experiments. The "6S" molecule found here would double the slope up to 12 per cent degradation in saturating enzyme conditions. Riley's finding that rapid isolation of RNA increases its apparent molecular weight and that 45S RNA, which has sequences of both 18S and 28S RNA, seems to have a molecular weight the same as 18S RNA, strongly indicates that the techniques he has used for RNA isolation may have led to some degradation of the RNA. Until his method is rigorously checked using denatured tRNA as a standard and alternative methods for RNA isolation, it is difficult to assess the validity of the exonuclease technique for determining molecular weights for RNA.

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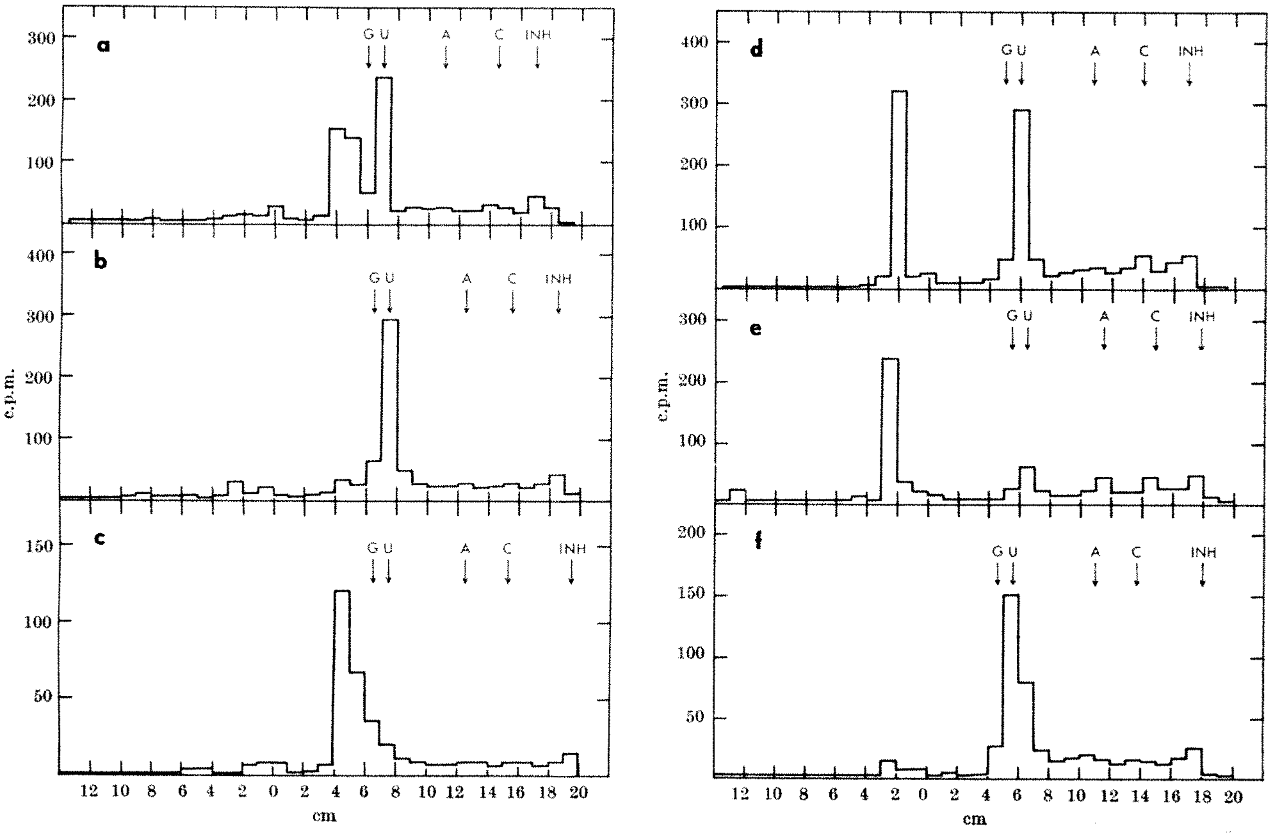

Fig. 2. Paper electrophoresis of pancreatic ribonuclease and ribonuclease T_1 digests of ^{3}H-isoniazid coupled RNA from various RNA fractions. Digestion and electrophoresis in 0·1 M triethylammonium formate (*p*H 3·0) were performed as described previously[4]. The paper was cut into 1 cm strips and moistened with water and either a dioxane scintillator[4] or the Beckman 'BBS3' toluene scintillator mixture added as described for Fig. 1. Counting efficiency with both systems was close to 19 per cent for tritium. (*a*)–(*c*) are ribonuclease T_1 digests, and (*d*)–(*f*) pancreatic ribonuclease digests. (*a*) and (*d*), untreated large ribosomal subunit RNA from peak II, Fig. 1 (*a*). (*b*) and (*e*) RNA heated in 0·5 per cent sodium dodecyl sulphate from peak III, Fig. 1 (*b*). (*c*) and (*e*) RNA heated in 0·5 per cent sodium dodecyl sulphate from peak I, Fig. 1 (*b*).

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Intergeneric Transfer of a β-Lactamase Gene between *Ps. aeruginosa* and *E. coli*

THE transfer of antibiotic resistance genes between strains of enteric bacteria by cell-to-cell contact was first detected[1] in 1959, and much effort has now been directed towards the understanding of this genetic process, particularly in relation to the ecology of man and his domestic animals[2-4]. It is now clear that the problems raised by this phenomenon are world-wide, and the recent publication of the Swann report[5], with its recommendations for the restriction in the use of antibiotics in animal husbandry, has also underlined many gaps in our knowledge. One of these is undoubtedly an understanding of the range of bacterial species that can accept antibiotic resistance genes by cell-to-cell contact and harbour the *R*-factors involved. We report here that antibiotic resistance markers, particularly for resistance to carbenicillin, can be transferred freely between strains of *Pseudomonas aeruginosa* isolated from burns, and various enteric bacteria.

Lowbury and his colleagues[6] have recently reported the isolation of a series of carbenicillin resistant strains of *P. aeruginosa* from burns. Preliminary evidence showed that the strains produced a β-lactamase but no attempt was made to characterize the enzyme(s) further. What we have found is that two of the strains isolated by Lowbury (1822 and 3425) produce a β-lactamase indistinguishable in properties from an enzyme commonly found in strains of enteric bacteria expressing *R*-factor-mediated resistance to penicillin, and that the genes responsible for the synthesis of this enzyme can be transferred from both the *Pseudomonas* strains to *E. coli* K12 by cell-to-cell contact.

The β-lactamase synthesized by strains 1822 and 3425 was examined in broken cell preparations by irradiating exponentially growing cultures of the strains in question at a density of about 1·0 mg dry weight organisms/ml. in a 60 W ultrasonic disintegrator at 25,000 Hz for 2·5 min. Disruption in these conditions caused about a four-fold increase in enzyme activity, and the specific enzyme activity of such broken-cell preparations was about 80 enzyme units/mg dry weight of original unbroken cells, measured with benzyl penicillin as substrate.

To identify the type of enzyme synthesized by *Ps. aeruginosa* strains 1822 and 3425, broken-cell preparations were examined for their relative rate of hydrolysis of a range of penicillin and cephalosporin substrates. These experiments suggested that the strains did not synthesize a β-lactamase of the type previously examined[7] and commonly found in *Pseudomonas* strains, nor of the type synthesized by *Ps. aeruginosa* strain Dalgleish[8,9], but that the enzyme was most similar to that isolated and purified from *E. coli* carrying the *R*-factor R_{TEM} (refs. 10 and 11). To examine this further, the substrate profile of broken cell preparations of *Pseudomonas* strains 1822 and 3425 was compared with semi-purified enzyme preparations from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$), from *Ps. aeruginosa* strain 1114 (a strain known to synthesize an enzyme indistinguishable from that described in *Ps. pyocyanea* NCTC 8203: refs. 7 and 9) and from *Ps. aeuruginosa* strain Dalgleish against benzylpenicillin, ampicillin, cephaloridine, cephalexin and carbenicillin as substrates. The profiles of these six preparations are shown in Table 1 and it seems clear that both strains 1822 and 3425 form an enzyme indistinguishable, on these grounds, from the enzyme synthesized by *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$), but quite different from the enzyme of *Ps.* strains 1114 and Dalgleish.

Table 1. SUBSTRATE PROFILES OF β-LACTAMASE FROM *Pseudomonas aeruginosa* STRAINS 1114, 1822, 3425, DALGLEISH AND FROM *Escherichia coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$)

			Substrate profile				
Strain No.	Species	Ref.	Benzyl penicillin	Ampicillin	Cephaloridine	Cephalexin	Carbenicillin
Test strains:							
1822	*Ps. aeruginosa*	6	100*	145	110	2	12
3425	*Ps. aeruginosa*	6	100*	157	125	2	10
Stock strains:							
1114	*Ps. aeruginosa*	6	100*	12	600	80	5
Dalgleish	*Ps. aeruginosa*	8, 9	100*	180	50	3	150
($\mathrm{R}^{+}_{\mathrm{TEM}}$)	*E. coli*	10, 11, 16	100*	165	125	2	12

* Arbitrary value of 100 for the rate of hydrolysis of benzyl penicillin.

The β-lactamase studied by Abraham and his colleagues in *Ps. pyocyanea* NCTC 8203 could be induced more than fifty-fold by growing the culture for about 3·5 h in the presence of 5 mg benzyl penicillin/ml.[7], and this behaviour was also found with Lowbury's strain 1114. By contrast, the enzyme from strains 1822 and 3425 (like that from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) (refs. 10 and 11)) was rigidly constitutive and neither growth in 5 mg benzyl penicillin/ml. nor growth in the same concentrations of ampicillin, cephaloridine or carbenicillin altered the rate of enzyme synthesis. To this extent, therefore, the synthesis of the β-lactamase by strains 1822 and 3425 was more similar to β-lactamase synthesis in *E. coli* than to synthesis in *Ps. aeruginosa* strain 1114.

The purification of the enzyme from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) in the past[11] has allowed specific antisera to this enzyme to be raised in rabbits, and it was already known that this antiserum was capable of neutralizing the β-lactamase against which it was prepared by about 60 per cent, but that the serum had no effect on the activity of enzyme preparations from *Pseudomonas* strains 1114 and Dalgleish[9]. Accordingly this serum was used to examine the enzymes from strains 1822 and 3425 in the hope that it might confirm the similarity between these enzymes and that of *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) that was implied by the substrate profile determinations described above. About 50 units of enzyme in a series of flasks was treated with an increasing quantity of antiserum (constant antigen titration) by the method described previously to study a similar interaction between staphylococcal exo-penicillinase and anti-staphylococcal penicillinase serum[12,13], and Fig. 1 shows the results obtained with various enzyme preparations. The enzymes from strains 1114 and Dalgleish were unaffected by the addition of antiserum (Fig. 1*d*, *e*), but the enzymes from strains 1822 and 3425 were neutralized to some extent by the serum (Fig. 1*b*, *c*) and the neutralization curves obtained were indistinguishable from that observed from the interaction of the serum with enzyme from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) (Fig. 1*a*, *b*, *c*).

To confirm the similarity of the enzymes from strains 1822 and 3425 with the enzyme from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$), crude enzyme preparations made by centrifuging broken-cell preparations of the strains in question were subjected to electrophoresis in starch gels prepared in borate

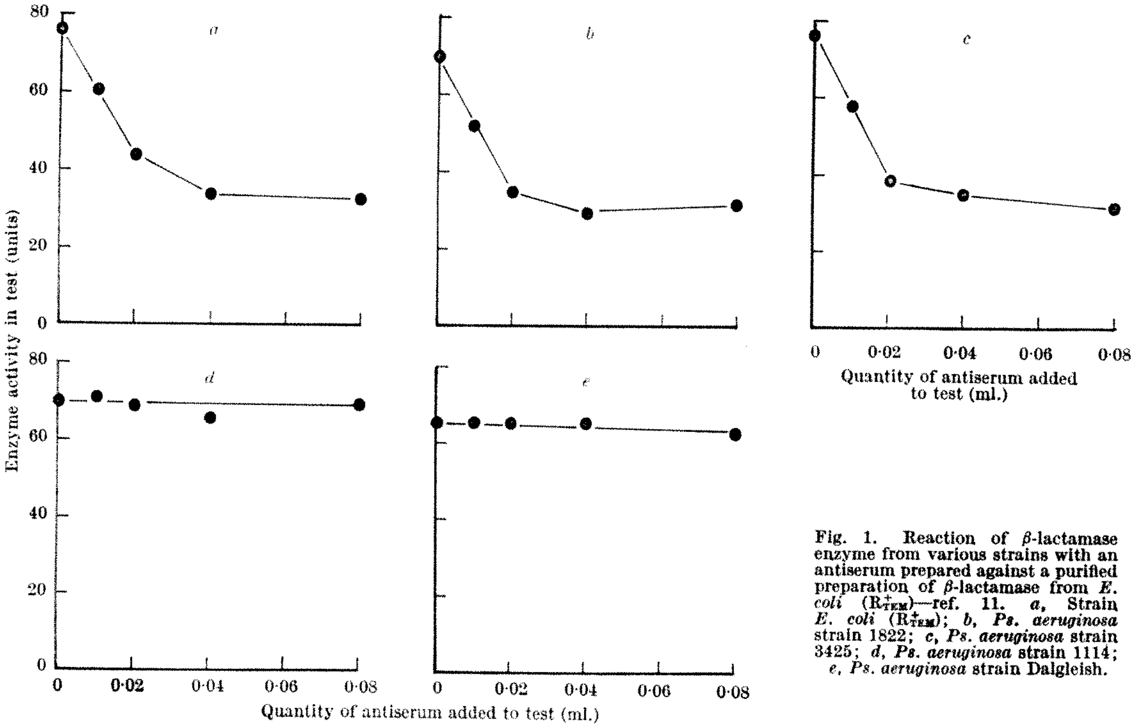

Fig. 1. Reaction of β-lactamase enzyme from various strains with an antiserum prepared against a purified preparation of β-lactamase from *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$)—ref. 11. *a*, Strain *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$); *b*, *Ps. aeruginosa* strain 1822; *c*, *Ps. aeruginosa* strain 3425; *d*, *Ps. aeruginosa* strain 1114; *e*, *Ps. aeruginosa* strain Dalgleish.

buffer, *p*H 8·5 (ref. 14). Once again the behaviour of the enzyme from *E. coli* and from *Ps. aeruginosa* strains 1822, 3425, 1114 and Dalgleish was compared. The gels were run in a potential gradient of 16 V/cm for 2·5 h and the location of the penicillinase detected by spraying the gels with a solution of 30 mg benzyl penicillin/ml. of 0·1 M Na_2HPO_4/KH_2PO_4 buffer, *p*H 5·9, containing 0·12 M KI and 0·016 M iodine[15,16]. The mobilities of the various enzyme preparations (Table 2) showed that the enzymes from strains 1822 and 3425 were indistinguishable from that of *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) but different from those obtained with the enzymes from strains 1114 and Dalgleish. The combination of similar substrate profile, antiserum neutralization curves and electrophoretic mobility makes it extremely likely therefore that the enzyme synthesized by *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$)—and also by many other strains of enteric bacteria carrying *R*-factors—is identical to that synthesized by *Ps. aeruginosa* strains 1822 and 3425.

The detection of an enzyme in *Ps. aeruginosa* strains 1822 and 3425 that is commonly *R*-factor-mediated in enteric bacteria suggests immediately that there may be an intergeneric gene transfer of this type of β-lactamase gene between *Ps. aeruginosa* and *E. coli*. Furthermore, this possibility is strengthened by the fact that Lowbury and his collaborators[6] reported that β-lactamase production can be "purged" from *Ps. pyocyanea* strains 1822 and 3425 by growth in acriflavine (an attribute of some extrachromosomal genetic elements similar to *R*-factors[17]) and by the reports[18,19] that *R*-factor transfer can occur, albeit at low efficiency, and under rather poorly specified conditions, between pseudomonads and various enteric bacteria. The transferability of the β-lactamase gene in *Ps. aeruginosa* strains 1822 and 3425 was investigated by the plate method of Lee and Richmond[20]. The donor culture was mixed with the recipient *E. coli* strain at a multiplicity of one donor/1,000 recipients and selection for transfer achieved by a combination of overlaying the cells on the agar with a solution of 50 μg ampicillin/ml. and then growing the cultures anaerobically in a modified Makintosh/Fildes jar. These experiments showed that these donor strains of *Pseudomonas* could transfer their β-lactamase genes to the *E. coli* K12 recipient, strain 1822 giving a transfer frequency of 1 : 10^4 and strain 3425 giving about 1 : 10^5. Examination of the type of enzyme synthesized by the *E. coli* recipients that have received the β-lactamase gene from *Pseudomonas* showed that the enzyme was indistinguishable, on the basis of substrate profile, from the enzyme synthesized by *E. coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$) (Table 3). Furthermore, the antibiotic resistance patterns of the *E. coli* strains that had received the lactamase genes in these experiments showed that all the cells had also received determinants conferring resistance to tetracycline and to neomycin and kanamycin.

The linked transfer of the β-lactamase, tetracycline resistance and kanamycin/neomycin resistance genes in these experiments suggested that the donor pseudomonads may be carrying an *R*-factor of the type usually found among enteric bacteria. To confirm that the gene transfer from these donor strains was by cell-to-cell contact and not by transduction, the rate of gene transfer on mixing donor and recipient populations was compared with the rate of transfer obtained when a culture filtrate from the donor culture was mixed with the recipient cells. The filtrate was prepared by taking the culture supernatant obtained by centrifuging a whole culture at 5,000*g* for 15 min and filtering it through a membrane with pore size

Table 2. MOBILITY OF VARIOUS β-LACTAMASE PREPARATIONS ON ELECTROPHORESIS IN STARCH-GEL AT *p*H 8·5

Strain No.	Species	Ref.	Mobility (cm/h)
Test strains:			
1822	*Ps. aeruginosa*	6	−1·0
3425	*Ps. aeruginosa*	6	−1·0
Stock strains:			
1114	*Ps. aeruginosa*	6	+0·3
Dalgleish	*Ps. aeruginosa*	8, 9	−0·6
($\mathrm{R}^{+}_{\mathrm{TEM}}$)	*E. coli*	10, 11, 16	−1·1

Table 3. SUBSTRATE PROFILES OF β-LACTAMASES FROM *Escherichia coli* ($\mathrm{R}^{+}_{\mathrm{TEM}}$), FROM *E. coli* ($\mathrm{R}^{+}_{seudomonas\ 1822}$) AND FROM *E. coli* ($\mathrm{R}^{+}_{Pseudomonas\ 3425}$)

Strain	Substrate profile				
	Benzyl penicillin	Ampicillin	Cephaloridine	Cephhalexin	Carbenicillin
Test strains:					
E. coli ($\mathrm{R}^{+}_{Pseudomonas\ 1822}$)	100*	155	127	2	10
E. coli ($\mathrm{R}^{+}_{Pseudomonas\ 3425}$)	100*	166	131	2	9
Stock strain:					
E. coli ($\mathrm{R}^{+}_{\mathrm{TEM}}$)	100*	160	125	2	12

* Arbitrary value for the rate of hydrolysis of benzyl penicillin.

capable of passing phage particles but of retaining any intact bacteria. Because gene transfer could not be detected using such filtrates (implying a frequency of transfer at least 10^5 smaller than obtained with cell–cell mixtures) it seems highly probable that this form of intergeneric gene transfer occurs by cell-to-cell contact.

Apart from the clinical importance of intergeneric transfer of this type, as was stressed by Lebek[18], the survival and effective functioning of the β-lactamase gene in two such dissimilar organisms are of great interest. Taxonomists have long separated Pseudomonads and the enteric bacteria on the grounds of their cultural characteristics, and a recent comparison between *Ps. aeruginosa* and *E. coli* using a comparison of 134 taxonomic characters in an Adansonian classification has shown that these species show a "similarity" (S-value) of only 35 per cent against values of 93 per cent or higher for two strains of *E. coli*[21]. Furthermore, the moles per cent G+C values for the total DNA of *Ps. aeruginosa* (64 per cent) are markedly different from that of *E. coli* (51 per cent)[22]. In short, therefore, one has here another example of the penetration of the gene pool of two widely different species of bacteria by a common extrachromosomal genetic element. Other examples of this phenomenon that have been reported include the transfer of *R*-factors from *E. coli* to *Salmonella* and to *Proteus* spp. as well as to *Serratia marcescens* (see ref. 23 for a review). In all these cases the phenotypic characters of the species involved have more in common than is the case with *E. coli* and *Ps. aeruginosa*. In practical terms, the intergeneric gene transfer of the type described here may mean that many genes, particularly those conferring resistance to antibiotics, that are usually encountered among the Enterobacteriacae may also occur among strains of *Ps. aeruginosa* and vice versa.

We thank Dr Edward Lowbury and his colleagues for giving us the strains we investigated, and for helpful discussions; Glaxo Laboratories, Ltd, for gifts of benzyl penicillin, cephaloridine and cephalexin; and Beecham Research, Ltd, for gifts of ampicillin and carbenicillin. The work was supported by the award of a postgraduate CAPS award to R. B. S. jointly by the Science Research Council and Glaxo Research, Ltd, and of a grant-in-aid from the Royal Society to M. H. R. for the purchase of a recording spectrophotometer.

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Enzymatic Binding of Valine to the 3' End of TYMV-RNA

THE genetic material of turnip yellow mosaic virus (TYMV) comprises RNA of molecular weight approximately 2×10^6, and of sedimentation constant close to 25S. In the presence of cell-free extracts and ATP, this RNA can interact with one amino-acid, valine, which becomes attached to its terminal nucleoside, an adenosine residue, by an ester linkage.

Table 1. REQUIREMENTS FOR THE BINDING OF VALINE TO TYMV-RNA

Conditions	^{14}C-Valine bound (pmoles)
Complete system	37
– Proteins	< 1
– ATP	< 1
– TYMV-RNA	< 1

The incubation mixtures contained in a final volume of 200 µl. were: 10 µmoles of Tris-HCl (*p*H 7·4), 1 µmole of $MgCl_2$, 0·2 µmole of DTT, 3 nmoles of ^{14}C-valine (125 mCi/mmole) and—where indicated—100 µg of *E. coli* proteins, 0·2 µmole of ATP and 50 pmoles of TYMV-RNA. After incubation for 30 min at 32° C, the reactions were stopped by addition of 5 per cent cold TCA and the resulting precipitates extensively washed on 'Millipore' filters and counted in Bray's solution[2]. *E. coli* (MRE 600) 105,000*g* supernatant was chromatographed on DEAE-cellulose to remove nucleic acids; the protein fractions eluted between 0·1 and 0·25 M KCl in 0·01 M Tris-HCl (*p*H 7·4) were used in these experiments. TYMV-RNA was stored at – 20° C as a precipitate in ethanol in the presence of bentonite.

The virus was isolated from the leaves of Chinese cabbage (*Brassica chinensis*) and its RNA extracted by phenol[1]. When this RNA was incubated with ^{14}C-valine, ATP and bacterial or yeast cell-free extracts devoid of endogenous RNA and free amino-acids, ^{14}C-valine was recovered with viral RNA. Table 1 shows the requirements of this reaction. The valine bound to TYMV-RNA was not removed by cold acid treatment (5 per cent trichloroacetic (TCA) or perchloric acid), was precipitated with the RNA by 1 M NaCl; it was, however, rapidly liberated at *p*H 10 or by hot TCA. Moreover, GTP, CTP and UTP did not promote this transfer, and at high concentrations these nucleotides inhibited the ATP-dependent reaction. Binding of valine and of other amino-acids to TYMV-RNA has been previously described by others[3], but with different results.

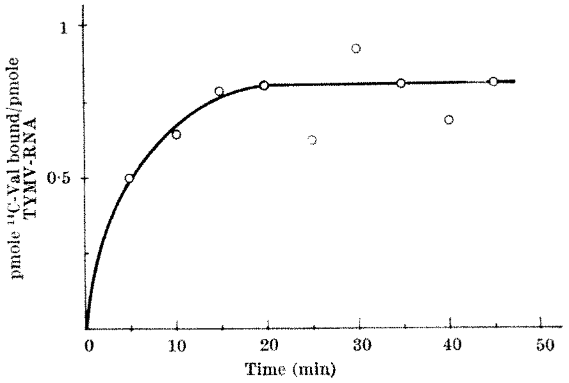

Fig. 1. Kinetics of valine binding to TYMV-RNA. The experimental conditions were as described in Table 1. At the times indicated, aliquots were removed and the radioactivity bound to cold TCA precipitated material was determined as already described.

We have followed the kinetics of valine binding to cold TCA precipitable material (Fig. 1). Maximum binding was at 15 min, and at this saturation point, 0·8 molecule of valine was bound per molecule of RNA (of mol. wt. 2×10^6). Using various batches of RNA stored for different periods of time, it was observed that this ratio fluctuated between 0·3 and 0·8.

Fig. 2 shows the behaviour of the charged RNA after centrifugation in sucrose gradients. About 80 per cent of the valine was recovered with 25S RNA, and the remaining 20 per cent was bound to slowly sedimenting material. On 'Sephadex G-100', in the presence or absence of 8 M urea, and at room temperature, about 75 per cent of the valine emerged in the void volume, and 25 per cent was retarded. The presence of $A_{260\ \mathrm{nm}}$ absorbing material closer to the top of the gradient (Fig. 2) and its absence in non-incubated RNA (not shown) proved that, during incubation, some degradation of TYMV-RNA occurred. TYMV-RNA treated at *p*H 10 to 11 is known to split into fragments sedimenting at about 5S (refs. 4 and 5).

To determine whether other amino-acids interacted similarly with this viral RNA, TYMV-RNA was incubated with a mixture of fifteen ^{14}C-labelled amino-acids (reconstituted hydrolysate of *Chlorella*); the radioactive material retained by the RNA was released by alkaline hydrolysis and analysed by thin-layer chromatography; valine was the only amino-acid recovered.

When charged TYMV-RNA was incubated for 1 h at room temperature in the presence of 0·5 M Tris-HCl (*p*H 8·2), and the radioactive material analysed by paper chromatography, valine and one other compound—identified chromatographically with Tris valyl-amide—were observed. The second compound must result from aminolysis of the bond between valine and TYMV-RNA by the NH_2 group of Tris. Identical results were obtained with valyl-tRNA of *E. coli* treated in the same conditions; O-methylvaline did not undergo aminolysis. The carboxyl group of valine bound to TYMV-RNA seems therefore to have become highly reactive, and probably forms an energy-rich ester bond. That the amino group of valine in valyl-RNA of TYMV is free was demonstrated by its capacity to be N-acetylated in the presence of acetic anhydride[6].

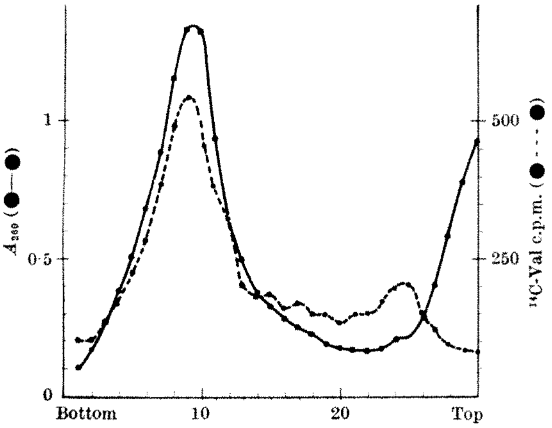

Fig. 2. Sedimentation pattern of ^{14}C-Val-RNA of TYMV. After incubation as described in Table 1, the charged RNA was isolated by the phenol procedure. It was layered over a 5–20 per cent sucrose gradient in 0·01 M sodium cacodylate (*p*H 6), and centrifuged in an 'SW 50' rotor for 3 h at 50,000 r.p.m. $A_{260\ \mathrm{nm}}$ readings were performed directly on the fractions collected; radioactivity was determined after cold TCA precipitation and filtration over 'Millipore'.

The half-life of valyl-RNA of TYMV was compared with that of valyl-tRNA of *E. coli* by incubation at *p*H 8·6: it was 60 min for both, which strongly suggests that in TYMV-RNA valine was esterified to the 2′ or 3′ position of the terminal nucleoside.

To determine the terminal nucleoside of TYMV-RNA which retained valine, we treated ^{14}C-Val-RNA by various nucleases and analysed the radioactive degradation products by thin-layer chromatography in different solvent systems (Fig. 3) and by paper electrophoresis; for comparison, ^{14}C-valyl-tRNA of *E. coli* was treated similarly. Both substrates yielded only valyl-adenosine or valyl-adenylate when treated by pancreatic ribonuclease or by snake venom phosphodiesterase, respectively. We conclude that valine is bound to the ribose moiety of adenosine on the 3′ end of the RNA.

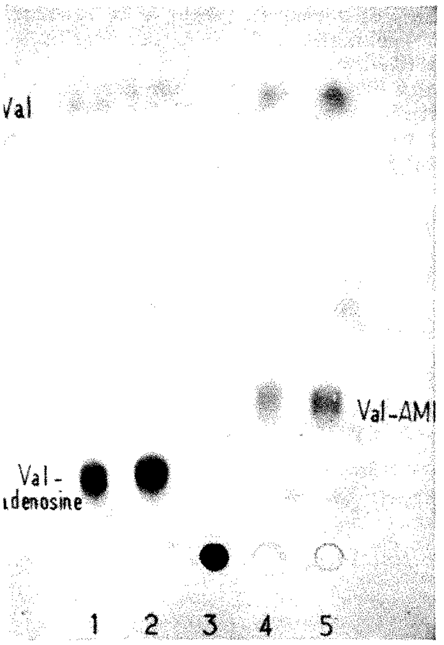

Fig. 3. Thin-layer chromatography of the radioactive products formed by nuclease digestion of ^{14}C-valyl-RNA of TYMV. ^{14}C-Valyl-RNA of TYMV was prepared as indicated in Table 1 and isolated by the phenol procedure; a sample of ^{14}C-valyl-tRNA of *E. coli* served as control. Each incubation mixture contained in 15 µl.; 50 pmoles of ^{14}C-valyl-RNA of TYMV (charged with 30 pmoles of ^{14}C-valine), or 600 pmoles of ^{14}C-valyl-tRNA of *E. coli* (charged with 30 pmoles of ^{14}C-valine), and either 2 µmoles of Tris-HCl (*p*H 7·4) and 5 µg of pancreatic ribonuclease, or 1 µmole of sodium-barbital–acetic acid (*p*H 8·6), and 25 µg of snake venom phosphodiesterase. After incubation for 20 min at 37° C, the samples were chromatographed on cellulose layer in a solvent containing ammonium sulphate (442 g/l.) : isopropanol (98 : 2) and autoradiographed. Treatments: (1) ^{14}C-valyl-RNA of TYMV plus pancreatic ribonuclease; (2) ^{14}C-valyl-tRNA plus pancreatic ribonuclease; (3) untreated ^{14}C-valyl-RNA of TYMV; (4) ^{14}C-valyl-RNA of TYMV plus snake venom phosphodiesterase; (5) ^{14}C-valyl-tRNA plus snake venom phosphodiesterase.

The identity of the bond joining valine to TYMV-RNA with that between valine and tRNA raises the question of the specificity of this viral RNA as acceptor of valine. The appearance of low molecular weight material (Fig. 2) suggests that valine could be bound to host tRNA trapped within the virus particle during the encapsidation process. This type of phenomenon is known to occur in the case of avian myeloblastosis virus[7,8]. In the case of TYMV-RNA, however, it seems improbable because a maximum of one valine molecule is bound per viral RNA, and no amino-acid other than valine is accepted. Moreover, when TYMV-RNA and ^{14}C-valyl-tRNA of Chinese cabbage were applied to the same sucrose density gradients, the analysis after centrifugation showed that valyl-tRNA had no detectable affinity for TYMV-RNA. A closer examination of the slowly sedimenting material of TYMV-RNA containing valine indicated that its sedimentation constant was somewhat higher than that of valyl-tRNA of Chinese cabbage, and must therefore originate from the degradation of TYMV-RNA during incubation. Although the possibility that a precursor of host $tRNA^{Val}$ might be strongly bound to TYMV-RNA cannot be excluded, the finding that 8 M urea does not dissociate them weakens this hypothesis.

Further results (unpublished) demonstrate certain similarities between $tRNA^{Val}$ and the TYMV polynucleotide moiety which accepts valine; this is shown by their interactions with three purified enzymes known to be specific for tRNA: tRNA. . .pCpCpA pyrophosphorylase, valyl-tRNA synthetase, and (after charging and acetylation) N-acyl-aminoacyl-tRNA hydrolase.

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Bone Marrow Transplantation in Irradiated Monkeys: Chromosome Aberrations in Donor Cells

SERIOUS chromosome injuries, of a type not previously reported, have been revealed unexpectedly in many donor cells during studies of bone marrow repopulation after transplantation to irradiated monkeys.

We studied baboons (*Papio hamadryas*) and rhesus monkeys (*Macaca mulatta*) exposed to ^{60}Co γ-irradiation in doses of 800 to 1,500 rads (dose rate, 50–200 rads/min). One and a half to two hours after irradiation, they received autologous or allogeneic bone marrow in doses of 2–5×10^8 viable nucleated cells per kg of body weight. In allotransplantation, females were used as donors and males as recipients. Because allogeneic bone marrow causes acute secondary disease in monkeys, with differentiation of haemopoietic cells only in a lymphoid direction and rapid "allergic death" of the immunocytes produced[1], this part of the experiment was done on monkeys thymectomized 6–12 weeks previously, in which the disease could not develop. The karyologic examination of bone marrow was made by the method of refs. 2 and 3.

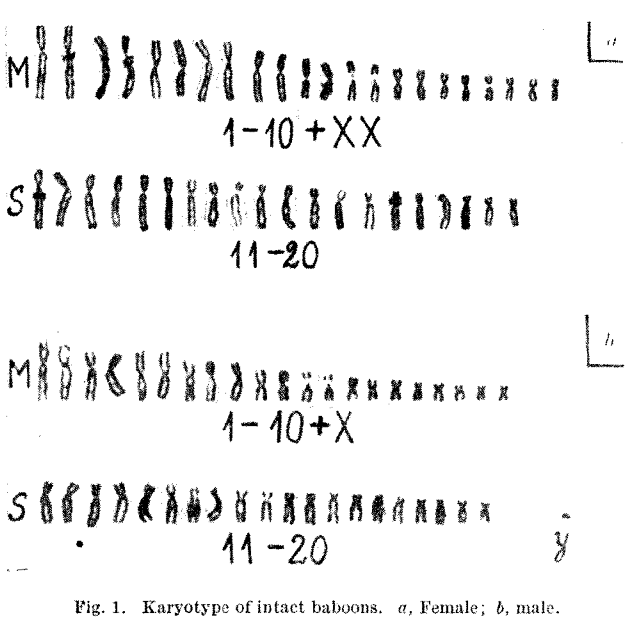

Fig. 1. Karyotype of intact baboons. *a*, Female; *b*, male.

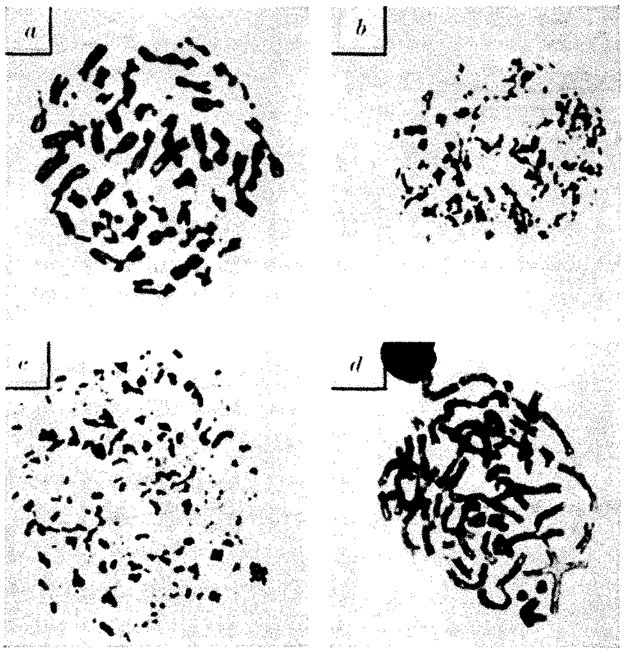

Fig. 2. Considerably injured cells from bone marrow of irradiated monkeys: *a*, numerous chromatid and isochromatid breaks (baboon; four days after exposure to 1,000 rads); *b*, *c*, pulverization (baboon; two days after exposure to 1,500 rads); *d*, breaks, chromatid translocations (baboon; four days after exposure to 1,500 rads).

The normal karyotype was studied on four baboons and two rhesus monkeys. The modal number of chromosomes in both species was 42. The cells of a male are easily distinguished from those of a female by the presence of a single acrocentric chromosome (*Y* chromosome) (Fig. 1). Aneuploid cells (about 5 per cent) were represented only by hypodiploids. In a series of 215 metaphases studied, gaps were found in three and a chromosome fragment in one. On the whole, the karyotype of the given monkey species was consistent with those described[4-6].

In the first 2–4 days after irradiation only considerably injured cells (Fig. 2) with numerous chromatid breaks and translocations were found in the monkeys; severe chromosome fragmentation (pulverization) was frequent. This was encountered in cells which had been exposed to the direct effect of ionizing radiation, for identical phenomena were revealed at the same time in control animals not given bone marrow. Active repopulation by the transplant occurred from the second week after irradiation (Table 1). Along with a few considerably injured cells a great number of uninjured metaphases belonging to the bone marrow donor (*XX*) were found, some of which had definite structural chromosomal abnormalities in the chromosomes. For analysis we accepted as identified only cells containing all forty-two chromosomes; those with even one chromosome lost or those with translocations were considered not identified. With such a strict approach all three monkeys were found to have chromosome aberrations in 7 to 30 per cent of cells of the bone marrow donor (Fig. 3*a*, *b*, *c*). Chromatid and isochromatid breaks were almost equal in frequency, whereas translocations were much rarer, as can be seen from Table 1.

Thus without taking into account the considerably injured cells which were apparently recipient cells fatally damaged by the direct effect of radiation, we found that 19 per cent of cells (63 of 327) carried structural chromo-

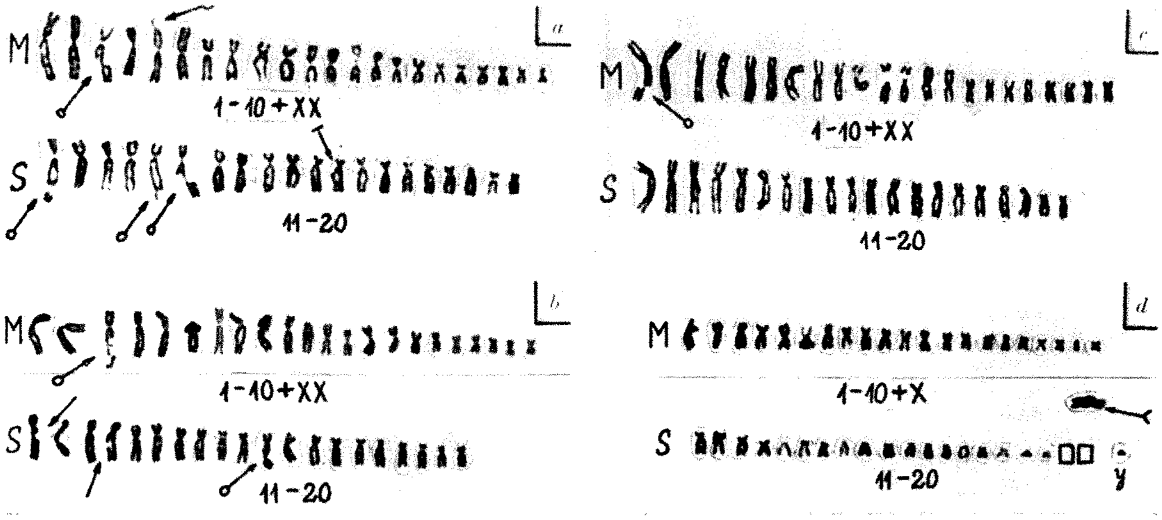

Fig. 3. Structural chromosomal abnormalities in haemopoietic cells of irradiated monkeys with transplanted bone marrow: *a*, baboon (*XY*) exposed to 1,000 rads and given bone marrow (*XX*) transplantation; eleven days after transplantation. Donor's cell: ○→, isochromatid break; |→, chromatid break. *b*, The same monkey fifteen days after transplantation. Donor's cell: ○→, isochromatid (chromosome) break; →, gap. *c*, Rhesus monkey (*XY*) exposed to 800 rads and given bone marrow (*XX*) transplantation; twelve days after transplantation. Donor's cell: ○→, isochromatid break. *d*, Baboon (*XY*) exposed to 900 rads and given transplantation of autologous bone marrow; 371 days after irradiation. >→, Triradial exchange figure; two chromosomes of group *S* are absent.

somal abnormalities; all identified aberrant cells (52 of 63) were donor's cells. An increase in the number of cells with gaps was also revealed among cells undergoing repopulation (12 of 327 compared with 3 of 215 in normal conditions); in some cases the gaps were numerous and much larger than those encountered in intact monkeys (Fig. 3*b*). By the end of the third postirradiation week the number of injured cells was reduced.

To exclude the influence of factors such as foreign antigenic surroundings, thymectomy of the recipient or possible allogenic inhibition, we conducted experiments with transplantation of autologous bone marrow (Table 2), and also revealed in such cases cells carrying similar chromosome aberrations. Because of the absence of markers we could not establish directly whether these cells belonged to the transplant. But with the irradiation dose used (1·5 to 2 LD_{99}) all cells which had been exposed to the direct effect of radiation and had started mitosis in the early postirradiation period carried serious chromosome injuries. It seems most probable that cells with relatively small injuries belonged to the transplant. This naturally does not refer to a monkey examined one and two years after irradiation. The two cells with chromatid translocations (Fig. 3*d*) found in it may have been the descendants of both irradiated and non-irradiated precursors. In these experiments, if greatly injured cells were not taken into account, in the first three weeks after transplantation 21 (14 per cent) of 148 cells carried chromatid and isochromatid breaks too and the number of cells with gaps (5 of 148) was also increased.

Two and a half months after irradiation the number of aberrant cells was reduced (1 of 35). Such a close resemblance between the relative numbers of aberrant cells following auto and allotransplantation suggests that in cases of autotransplantation these injuries are also carried by grafted cells.

The chromosome injuries encountered with both allo and autotransplantation were not self-reproducing and were lost at the first mitosis; no clones of injured cells were found. This suggests that the damage is incurred not only during transplantation or soon after it. Indeed, in some cases 15 to 20 per cent of all mitotic donor cells were aberrant 12 to 15 days after transplantation. Because the cellularity of the bone marrow was restored to a

Table 1. CHROMOSOME ANALYSIS OF MALE MONKEYS AFTER TRANSPLANTATION OF FEMALE BONE MARROW

Monkey species and irradiation dose	Post-transplantation day	No. of metaphases analysed	Cells with chromosome No.			No. of cells with structural chromosomal abnormalities*				No. of cells with gaps*
			< 42	42	> 42	Greatly injured	Chromatid breaks	Isochromatid breaks	Translocations	
Baboon, 1,000 rads	2	20				20/0				
	6	0								
	8	30	1	28	—	1/0	2/2	7/6	—	2/2
	11	100	5	67	2	26/0	15/14	6/5	3/0	5/5
	15	50	1	43	—	6/0	4/4	5/4	—	2/2
	21	50	2	45	1	2/0	2/1	4/4	1/0	1/1
	24	10	—	10	—	—	—	—	—	—
Rhesus, 800 rads	12	70	5	62	—	3/0	7/7	3/2	1/0	2/2
Rhesus, 800 rads	12	55	10	45	—	—	2/2	1/1	—	—
Total		385	24	300	3	58/0	32/30	26/22	5/0	12/12

* The numerator shows the total number of cells; the denominator, the number of identified donor cells.

Table 2. CHROMOSOME ANALYSIS OF IRRADIATED MONKEYS AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION

Monkey species and irradiation dose	Post-transplantation day	No. of metaphases analysed	Cells with chromosome No.			No. of cells with structural chromosomal abnormalities				No. of cells with gaps
			< 42	42	> 42	Greatly injured	Chromatid breaks	Isochromatid breaks	Translocations	
Baboon, 1,500 rads	4	1	—	—	—	1	—	—	—	—
	8	0	—	—	—	—	—	—	—	—
	9	21	2	18	—	1	—	1	—	—
Rhesus, 800 rads	21	100	4	94	—	2	6	9	—	4
Rhesus, 800 rads	21	34	1	32	1	—	—	1	4	1
	74	35	1	34	—	—	1	—	—	1
Baboon, 900 rads	371	33	1	32	—	—	—	—	1	1
	654	100	8	92	—	—	—	—	1	—
Total		324	17	302	1	4	7	11	6	7

great extent by that time (it reached 15,000 to 30,000 per mm^3 as compared with 200 to 1,000 per mm^3 six days after irradiation at the depth of aplasia), the total number of injured cells exceeded the total number of transplanted cells capable of prolonged proliferation.

From this it can be concluded that factors which can cause chromosome aberrations in the proliferating haemopoietic cells exist in the body during the first weeks after irradiation. There are no facts pointing to this type of activity in "radiotoxins". It seems possible that injuries to donor cells may be caused by a virus infection which develops frequently in monkeys suffering the severest form of acute radiation sickness. Whatever the mechanism of such injuries, however, the results should be taken into account in radiobiological studies. They suggest in particular that not every cell with chromosome aberrations found in an irradiated animal has been exposed to the direct action of radiation.

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Evidence against a Presynaptic Action of Acetylcholine during Ganglionic Transmission

THE receptors for the neurotransmitter in sympathetic ganglia (acetylcholine) are usually thought to be located exclusively on the postsynaptic membrane. Koelle[1,2] has suggested, however, that the presynaptic nerve terminals are also sensitive to acetylcholine, and that, during normal transmission, the acetylcholine initially released by the preganglionic nerve impulse might depolarize the terminals and release a further quantity of acetylcholine, so amplifying the transmitter release process. Koelle recognized that this hypothesis was similar to that advanced in connexion with transmitter release from adrenergic fibres[3], and suggested that such "cholinergic links" may be a widespread feature of neurotransmitter and neurosecretory systems.

At present, however, experimental evidence for the presence of presynaptic acetylcholine receptors in ganglia is inconclusive. The principal evidence originally advanced was that the effect of carbachol (a stable analogue of acetylcholine) on the cat superior cervical ganglion was depressed following degeneration of the preganglionic nerves[4], from which it was concluded that the stimulant action of carbachol on normally innervated ganglia results principally from the liberation of acetylcholine from the preganglionic fibres. The force of this argument is diminished, however, by the failure of subsequent investigations to confirm denervation subsensitivity in ganglia (refs. 5, 6 and personal communication from R. L. Volle).

A more direct approach would be to find out if application of acetylcholine could release acetylcholine from the preganglionic fibres. Some experiments along these lines on the perfused cat superior cervical ganglion indicated that carbachol could release acetylcholine, but the mode of action of carbachol differed radically from that of preganglionic nerve stimulation: thus, unlike the response to nerve stimulation, the effect of carbachol was not prevented by omitting Ca^{2+} from the perfusion fluid, yet was reduced by curare alkaloids, hexamethonium and atropine[7,8]. In view of these differences, the relevance of the effect of carbachol to the postulated action of neurally released acetylcholine on the presynaptic terminals is questionable. For this reason further experiments of this nature—but using acetylcholine itself as the stimulant agent—seemed necessary, and have been undertaken in the present study.

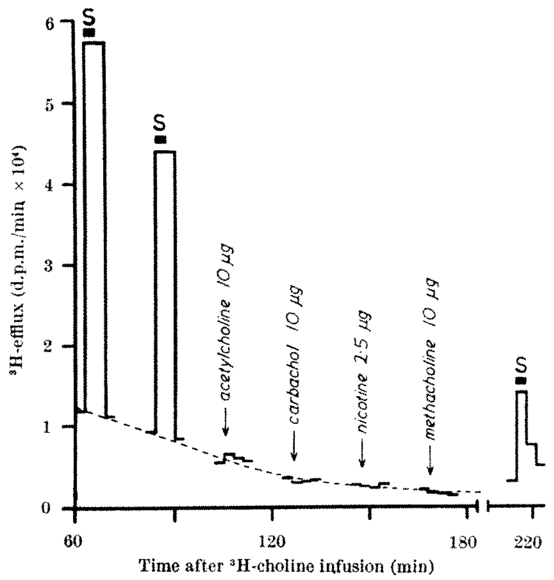

Fig. 1. Effects of preganglionic nerve stimulation (S = 3 min at 10 Hz) and injections of cholinomimetic agents (in 0·1 ml. Ringer–Locke) on the efflux of tritium from a perfused cat superior cervical ganglion. Efflux into unlabelled perfusion fluid was measured, the ganglionic acetylcholine having been previously labelled by perfusing with ^{3}H-choline solution while stimulating the preganglionic nerve trunk (see text). The discontinuities in the efflux curve were occasioned by the intermittent blood perfusion[11].

The chief difficulty presented by such experiments is that of detecting the small amounts of acetylcholine released from the nerves in the presence of much larger quantities injected: for this, conventional bioassay techniques are clearly unsuited. Instead, we have labelled the neural acetylcholine stores radioactively, by infusing radioactively labelled choline while stimulating the preganglionic nerve. Then some of the unlabelled acetylcholine originally present is released and replaced by labelled acetylcholine, formed from the labelled choline[9]. Release of neural acetylcholine may then subsequently be detected by radioassay.

The experimental procedure was as follows. Cats were anaesthetized with chloralose (70 mg/kg intravenously) after induction with halothane. The superior cervical ganglion was perfused with oxygenated Ringer–Locke solution containing physostigmine (10 μg/ml.), as described by Perry[10], but modified in that the cat's own blood was passed intermittently through the ganglionic circulation to reduce ganglionic oedema[11]. To label the neural acetylcholine stores, ^{3}H-choline (15 μCi, 1 μg/ml.; Radiochemical Centre) was added to the perfusion fluid and perfused through the ganglion for 15 min while stimulating the preganglionic nerve trunk at 10 to 50 Hz (0·5 ms duration, 10 V). The perfusion fluid was then changed to Ringer–Locke solution containing unlabelled choline for the remainder of the experiment, and total radioactivity in the effluent perfusate monitored by liquid scintillation counting. To separate ^{3}H-choline and ^{3}H-acetylcholine in the perfusate, samples of perfusate were freeze-dried, choline and acetylcholine were dissolved in ethanol, and the ethanol solution was subjected to paper electrophoresis[12]. Choline and acetylcholine bands were located by iodoplatinate spray, cut out, extracted into phosphor, decolorized with NH_4OH, and radioactivity was counted. A single run sufficed to remove 98–99 per cent of choline from acetylcholine. Throughout the experiment, ganglionic responses to preganglionic nerve stimulation, and to drug injections (administered into the influent perfusion stream in a volume of 0·1 ml. warmed Ringer–Locke), were monitored by recording the contraction of the nictitating membrane.

Stimulation of the preganglionic nerve trunk at 10 Hz while perfusing with unlabelled choline solution regularly increased the amount of tritium in the effluent perfusate (Fig. 1). Separate measurement of acetylcholine and choline in the perfusate showed that this increased radioactivity arose entirely from an increased level of ^{3}H-acetylcholine. The amounts of ^{3}H-acetylcholine released declined with successive periods of nerve stimulation, whereas the total acetylcholine released (measured by bioassay on the rat blood pressure) remained constant. This fall in ^{3}H-acetylcholine specific activity, which was an exponential function of the number of nerve impulses, was presumably the result of replacement of ^{3}H-acetylcholine released by unlabelled acetylcholine synthesized from the unlabelled choline in the perfusion fluid—that is, the reverse of the labelling procedure. By extrapolating the specific activity of the released ^{3}H-acetylcholine to zero impulses, a rough estimate of the original specific activity of the releasable ^{3}H-acetylcholine could be obtained: this was between 25 and 75 per cent of that of the ^{3}H-choline infused.

Acetylcholine was injected into the perfusion stream in nine experiments (seventeen injections), in doses from 5 to 100 μg in 0·1 ml. Ringer–Locke. These injections produced contractions of the nictitating membrane which were frequently as great as, and sometimes greater than, those elicited by preganglionic nerve stimulation. Nevertheless, acetylcholine injections did not release appreciable amounts of ^{3}H-acetylcholine. In most instances (Fig. 1) the total radioactivity in the effluent perfusate was hardly altered following such injections. When total radioactivity was increased (five injections), it was found to result, not from a selective increase in ^{3}H-acetylcholine, but from an increased efflux of ^{3}H-choline. In such cases, the acetylcholine injection greatly increased the perfusion flow rate through the ganglion, and also increased the efflux of extracellular marker substances (^{14}C-sucrose or $Na_2{}^{35}SO_4$). This suggests that the ^{3}H-choline was derived from extracellular rather than intraneuronal sources.

Carbachol, nicotine, tetramethylammonium and methacholine were no more effective than acetylcholine in releasing ^{3}H-acetylcholine. Like acetylcholine, carbachol and methacholine (both effective ganglionic vasodilators) sometimes increased ^{3}H-choline efflux. Because methacholine did not stimulate the ganglion, this effect was clearly unrelated to ganglion stimulation, as indeed the observations with acetylcholine suggest.

Injection of KCl augmented the release of ^{3}H-acetylcholine, but only in concentrations (120 mM) three times greater than that which produced a maximal postganglionic response. It seems that, even with this non-specific stimulant, the postganglionic response cannot be ascribed to any material extent to release of acetylcholine.

Full interpretation of these results depends to some extent on the homogeneity of the ganglionic acetylcholine stores. There is evidence that a small fraction of the preganglionic acetylcholine (about 15 per cent) is not normally available for release by the nerve impulse[10,13]. In addition to this "reserve" acetylcholine, when a ganglion is perfused with physostigmine solution (as in the present experiments), a further and larger pool of "surplus" acetylcholine is formed[13]. This also does not directly contribute to the transmitter released by the nerve impulse[13], though it probably exchanges slowly with the releasable fraction[14,15]. Thus, during the relatively short period of perfusion with ^{3}H-choline, it is possible that neither the reserve nor surplus stores were appreciably labelled. In this case, if the injections of acetylcholine had released acetylcholine from these pools, this might not have been detected by radioassay.

Release of unlabelled acetylcholine is, however, irrelevant to the question whether neurally released acetylcholine exerts a presynaptic action, for acetylcholine pools not labelled during preganglionic nerve stimulation clearly do not directly participate in the transmission process. Our significant finding is that injections of acetylcholine were unable to release ^{3}H-acetylcholine, that is, to release acetylcholine from the pool discharged by the nerve impulse. This is strong evidence that a presynaptic action of acetylcholine does not play any part in the transmission process. Of course, it might be argued that the concentration of neurally released acetylcholine available to the hypothetical presynaptic receptors is greater than that attainable by injection—perhaps through some "barrier" to extrinsic acetylcholine. Such arguments are difficult to counter, though the substantial postsynaptic effects of the injections make the barrier concept rather unlikely. It would seem more reasonable to conclude that the neurotransmitter at the ganglion acts exclusively on the postsynaptic membrane.

During the course of this study observations were briefly reported[16] which are in agreement with our findings.

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Definitive Evidence for the Short Arm of the *Y* Chromosome associating with the *X* Chromosome during Meiosis in the Human Male

MEIOTIC studies on the human male during the past thirty years, notably those of Koller[1] and Ford and Hamerton[2], have shown that the *X* and *Y* chromosomes associate at meiotic prophase. Whether the chromosomes form a true chiasma or not has never been demonstrated convincingly. Because the position of the centromeres of the chromosomes cannot be identified with certainty in meiotic preparations, there has been much speculation as to whether it is the long or the short arms of the *Y* chromosome which associate with the *X*. Thus, Sasaki and Makino[3] suggested that the *X–Y* chromosome association involved the short arms of the *Y*, and Hultén *et al.*[4] suggested the long arms of the *Y*. The best cytological evidence has come from McIlree *et al.*[5] on a male with a dicentric *Y* chromosome involving a presumptive deletion of the short arms. At diakinesis the dicentric *Y* chromosome did not associate with the *X*, and Jacobs therefore suggested that in normal circumstances the *Y* chromosome might have associated with the *X* by its short arms[6]. From phenotype–karyotype correlations, Ferguson-Smith[7,8] deduced that part of the long arms of the *Y* chromosome was homologous with part of the short arms of the *X*, and that this region was involved in the *X–Y* association. From the appearance of the *X–Y* association at diakinesis in testicular biopsies from a population of normal males, we have tentatively concluded that the short arms of the *Y* chromosome are involved in associating with the *X* chromosome.

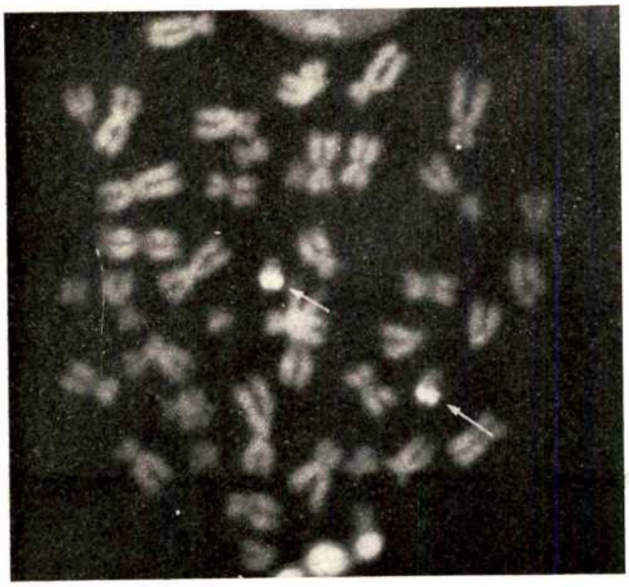

Fig. 1. Mitosis in a human male with *XYY* sex chromosomes showing a highly fluorescent distal region in the long arms of the *Y* chromosomes.

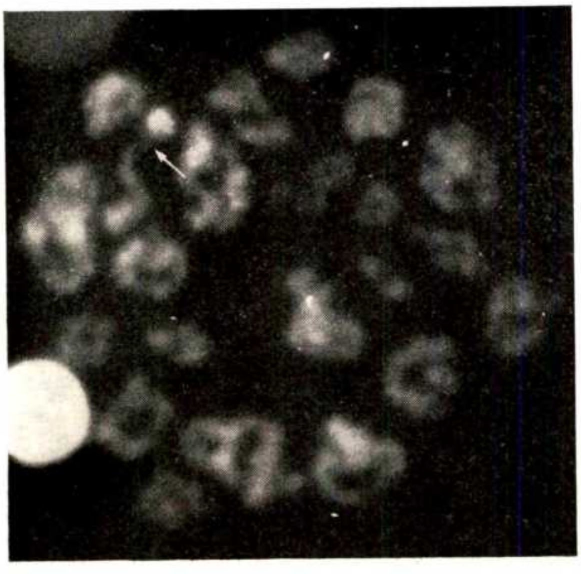

Fig. 2. **Diakinesis showing a highly fluorescent region at the extreme end of the *X–Y* bivalent in a normal male.**

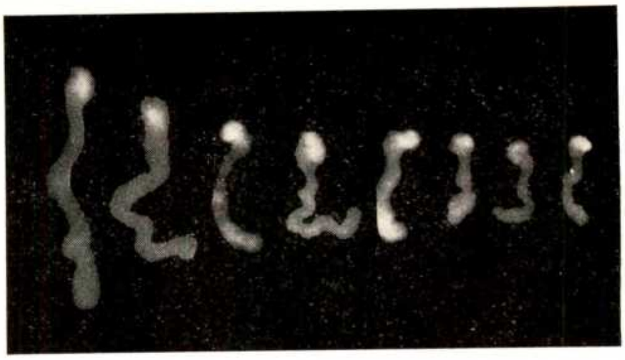

Fig. 3. Sex bivalents from different cells showing a fluorescent region at one end of each bivalent.

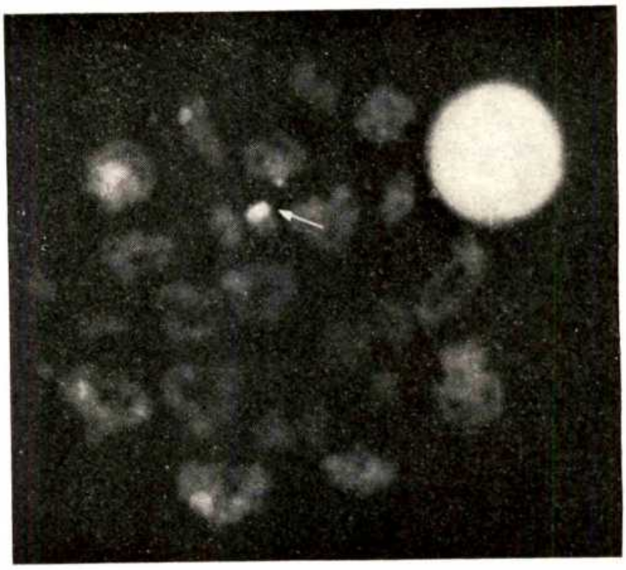

Fig. 4. Diakinesis showing a separate *Y* with a highly fluorescent and a low fluorescent region.

Zech[9] has demonstrated that the distal ends of the long arms of the human *Y* chromosome at mitosis fluoresce when stained with quinacrine mustard. The same effect can be achieved with quinacrine dihydrochloride[10] (Fig. 1) and the fluorescent *Y* chromatin can also be detected in interphase nuclei[11]. This can be used for determining the number of *Y* chromosomes present in the resting state nuclei of buccal mucosa cells. Moreover, the distal ends of the long arms of the *Y* chromosome fluoresce in meiotic cell preparations throughout all stages from spermatogonia through to mature spermatozoa[13]. We examined diakinetic cells prepared according to the technique of Evans *et al.*[12], stained them with quinacrine dihydrochloride[11] and viewed them with a Leitz microscope fitted with an HBO 200 light source and a 'Ploem' vertical illuminator. Fifty cells were selected from a normal male on the basis that they showed differential fluorescence and the *X–Y* bivalent was examined under ultraviolet light and under phase contrast. In all cases where the *X* and *Y* chromosomes were associated, the more highly fluorescent region was situated right at one end of the sex bivalent (Figs. 2 and 3) and not in an interstitial position. Where the *X* and *Y* chromosomes were lying free, the *Y* chromosome characteristically had a highly fluorescent area and a less fluorescent area (Fig. 4), thus mirroring the staining distribution in the *Y* chromosome during mitosis. It seems reasonable to suppose that the short arms, centromere and proximal parts of the long arm are situated in the less fluorescent part of the chromosome as they are in the mitotic *Y* chromosome.

We interpret these findings as definite evidence that it is the short arm of the Y chromosome which associates with the X during the first meiotic prophase.

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The *Y* Chromosome in Human Spermatozoa

ATTEMPTS have been made to distinguish between spermatozoa of mammals containing either an X or a Y chromosome by looking for differences in size and surface properties of the two classes of cells (see refs. 1 and 2 for reviews), but the results of such approaches are equivocal. Another approach is to try to identify the X or Y chromosome within the head of the spermatozoon. Like Zech[3] and others[4,5], we have found that the distal end of the long arm of the Y chromosome in mitotic and meiotic metaphase nuclei of human cells displays a fluorescence more brilliant than that shown by the other chromosomes when stained with either quinacrine or quinacrine mustard. This property is carried by the Y chromosome into the chromatin of the mature spermatozoon.

Human spermatozoa were smeared on slides, dried in air, washed in absolute ethanol for 5 min and then stained for 20 min in 0·5 or 1 per cent quinacrine or 0·005 per cent quinacrine mustard dissolved in deionized water. The slides were thoroughly washed and the preparations mounted in water with a coverslip sealed down with varnish. The preparations remain in good condition for some hours. Spermatozoa were viewed with fluorescent illumination from a Zeiss photo-microscope using exciter filters with transmission ranges between 330 and 500 nm.

Chromatin in the heads of spermatozoa fluoresces with a yellow-green colour. The basal third of the head shows a denser fluorescent mass than the remaining upper portion and there is a sharp boundary between the two regions. Some sperm show a fluorescent body (F-body) that is much brighter than the bulk of the fluorescent material (Fig. 1). This F-body we believe to be the chromatin in the distal segment of the long arm of the Y chromosome. Spermatozoa stained with 0·05–0·5 per cent acridine orange, thought to intercalate into the DNA helix in the same way as quinacrine[6], display no F-bodies. Neither does ethidium bromide—a fluorochrome that shows the reverse pattern to quinacrine fluorescence with some types of heterochromatin[7]—differentiate the Y chromosome from other chromosomes in mitotic or sperm nuclei. The frequency of the F-body in spermatozoa of similar morphology was estimated (Table 1). Nearly half the quinacrine mustard-stained spermatozoa had an F-body, but it is seen less frequently after quinacrine staining. The lower frequency is probably caused by fading of the fluorescence with prolonged viewing, making detection of the F-body difficult. Fluorescence from quinacrine mustard fades much less. If the F-body is indeed chromatin of the Y chromosome it might be expected to be present in 50 per cent of the spermatozoa. The ratio may be lowered by the difficulty of resolving the F-body when it lies within the dense chromatin at the base of the head. Occasionally a spermatozoon was seen with two F-bodies; these spermatozoa had two tails and so may be diploid.

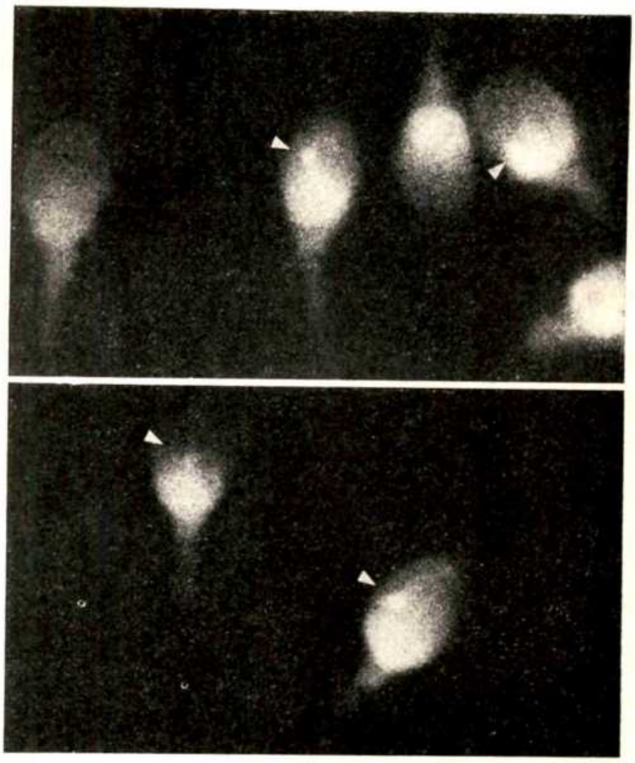

Fig. 1. A group of quinacrine-stained spermatozoa showing fluorescence. Some have a brightly fluorescent F-body (arrowed); others lack the F-body. × 4,300.

Table 1. FREQUENCY (PERCENTAGE) OF SPERM IN WHICH AN F-BODY IS VISIBLE (+) OR INVISIBLE (−)

Fluorochrome	Sample	+	−	No. of observations
Quinacrine mustard	1	46·5	53·5	200
	2	44·4	55·6	367
	1+2	45·1	54·9	567
Quinacrine	1	38·5	61·5	109
	2	40·6	59·4	404
	1+2	40·2	59·8	513

In more than 60 per cent of the spermatozoa with an F-body this was seen to lie at the boundary between the dense and less dense regions of chromatin (Fig. 1). In the others the F-body lay with equal frequency in either the dense or less dense regions. In some spermatozoa the F-body could be seen to lie against the membrane of the head.

We believe that the F-body represents the Y chromosome for the following reasons. In mitotic and meiotic metaphases part of the Y chromosome fluoresces more than any other chromosome; in interphase nuclei of normal XY males there is only one strongly fluorescent spot of chromatin, while in nuclei from XYY males there are two spots; nuclei of normal XX females exhibit no such spots[4]. In spermatozoa the frequency with which F-bodies are seen approaches that expected from the segregation of the Y chromosome at meiosis. We presume that spermatozoa lacking an F-body lack a Y chromosome and therefore may be X-bearing sperm.

The regular positioning of the F-body in the sperm head is of interest, for other authors have found an ordered arrangement of chromosomes in sperm[8–10]. There is, however, nothing which yet suggests structural differentiation of the Y chromosome within the sperm head when sperm chromatin is examined with the electron microscope[11–14].

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Membrane Noise produced by Acetylcholine

ELECTROPHYSIOLOGICAL studies of the motor end-plate have shown that there is intermittent release of multimolecular ("quantal") packets of acetylcholine (ACh) from the nerve terminals, resulting in the appearance of discrete miniature end-plate potentials (m.e.p.p.). The number of molecular reactions which summate to make up a single m.e.p.p. is not known; it must be very large, for—with minimal doses of applied ACh—the membrane response seems to be continuously graded, even at amplitudes which are one or two orders of magnitude below that of the m.e.p.p. (see, for example, ref. 1, page 10).

It seemed possible, however, that during steady application of ACh to a motor end-plate the statistical effects of molecular bombardment might be discernible as an increase in membrane noise, superimposed on the maintained average depolarization. Suppose, for instance, that a maintained ACh potential V is made up of a statistical fusion of many "elementary" effects, each of instantaneous amplitude a and exponential decay with membrane time constant τ. Whether the elementary event arises from the reaction of one or more ACh molecules, with one or several receptor molecules, is left open. The average rate of such elementary effects necessary to maintain the depolarization V is then given by $n = V/a\tau$. For example, if $a = 0{\cdot}1\ \mu V$ and $\tau = 10$ ms, then to produce a 10 mV depolarization requires an average frequency of 10^7 elementary events per second. Applying Rice's theory of random noise[2] to this case, it can be shown that the expected ACh-induced voltage fluctuations across the membrane would have a root mean square value E of approximately $\sqrt{Va/2}$, which, for the example quoted, is about 22 µV. Conversely, knowing V and E, the elementary voltage amplitude a can be derived.

Fig. 1 shows that ACh-induced membrane noise can, in fact, be detected. We have used either bath application of ACh or, more conveniently, local application to a single end-plate by allowing ACh to diffuse from a micropipette (placed at sufficient distance to render ineffective any possible fluctuations in position or resistance of the pipette). Membrane potential changes during ACh application were recorded simultaneously on a low gain d.c. and a high gain a.c. coupled channel (time constant approx. 0·1 s). In the experiment illustrated in Fig. 1, a maintained depolarization of 8·5 mV was accompanied by membrane noise of 29·2 µV (root mean square value). The calculated value of a is 0·2 µV. Making an approximate allowance for non-linear summation of ACh potentials[3], the corrected values of V, E and a are 9·7 mV,

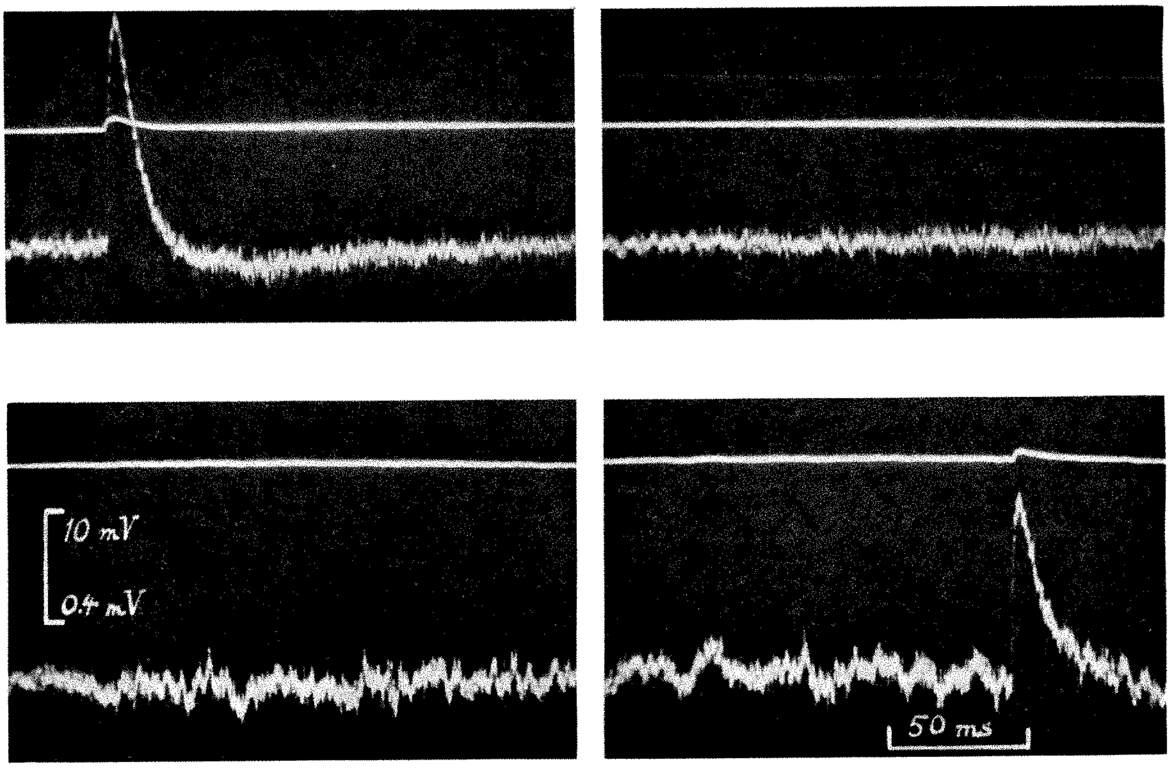

Fig. 1. Intracellular recording of membrane potential from end-plate region of frog muscle fibre. In each block, the upper trace was recorded on a low gain d.c. channel (10 mV scale); the lower was simultaneously recorded on a high gain a.c. coupled channel (0·4 mV scale). The records in the upper row are controls (no ACh); the lower row shows membrane noise during ACh application, by diffusion from a micropipette. In the lower records, the increased distance between a.c. and d.c. traces shows upward displacement of the d.c. trace because of ACh-induced depolarization. Two spontaneous m.e.p.p.s are also seen.

37·8 μV and 0·29 μV respectively. The average m.e.p.p. in this fibre (in presence of neostigmine) was 0·7 mV. Several experiments of this kind have been performed showing that the average amplitude of the m.e.p.p. is a few thousand times greater than the "elementary" ACh effect. It should be noted that the membrane noise described here accompanies ACh-induced depolarizations, but that no comparable effect was found during an electrotonic potential change of similar size, produced by passing direct current across the fibre membrane. We suggest that the membrane noise observed in these experiments arises from statistical variation of high-frequency collisions between ACh molecules and end-plate receptors.

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Hypothalamic β-Adrenergic "Satiety" System antagonizes an α-Adrenergic "Hunger" System in the Rat

THIS communication proposes and tests the hypothesis that in the central nervous system, specifically in the hypothalamus, α and β-receptors function antagonistically in an adrenergic system, to regulate hunger in the rat. More specifically, this antagonistic relationship takes place between a hypothalamic α-adrenergic "hunger" system, which elicits eating behaviour, and a hypothalamic β-adrenergic "satiety" system, which suppresses eating behaviour. Before this research, there has been no evidence for a central β-adrenergic system functioning antagonistically with respect to a central α-adrenergic system.

Peripheral injections of three adrenergic agonists, norepinephrine, epinephrine or isoproterenol, have been shown to suppress food intake (refs. 1 and 2 and unpublished work of W. Goldman and D. Lehr). These anorexic effects, at least when induced by epinephrine and isoproterenol, have been partially blocked by a peripherally injected β-adrenergic antagonist (ref. 2 and unpublished work of W. Goldman and D. Lehr). On the other hand, in the central nervous system, studies using the technique of cannula-guided chemical stimulation of the brain, have shown that hypothalamic injections of norepinephrine or epinephrine increase food intake in satiated rats (refs. 3 and 4 and unpublished work of S. F. Leibowitz). Moreover, these eating effects were blocked by a centrally injected α-adrenergic antagonist (ref. 4 and unpublished results of S. F. Leibowitz). These results from peripheral and central studies formed the experimental background for our research on the brain. The main objectives of this research were to demonstrate the existence of a central β-adrenergic system, to confirm the existence of a central α-adrenergic "hunger" system, and, most significantly, to show that the antagonistic interaction between a central α and β-adrenergic system is a key mechanism in the regulation of hunger/satiety in the rat.

To investigate these hypotheses, we made the following predictions, and tested them in the hypothalamus. (1) If the eating response is mediated by hypothalamic α-adrenergic receptors, it should be elicited by a hypothalamically injected α-adrenergic agonist. (2) If the anorexic response is mediated by hypothalamic β-adrenergic receptors, it should be elicited by a hypothalamically injected β-adrenergic agonist. (3) This β-mediated anorexic response evoked by a β-adrenergic agonist should be blocked by a β-adrenergic antagonist but not by an α-adrenergic antagonist. (4) When an adrenergic agonist with both α and β-receptor activity evokes as its dominant response the β-mediated anorexic response, and when this particular agonist is combined with a β-adrenergic antagonist, the latent α-mediated eating effects of this agonist, previously masked by the dominant β-mediated anorexic effects, should now become apparent. (5) The β-mediated anorexic response evoked by an adrenergic agonist should be potentiated by an α-adrenergic antagonist. Evidence for the last two predictions would demonstrate most clearly the antagonistic interaction between central α and β-receptor control of eating behaviour.

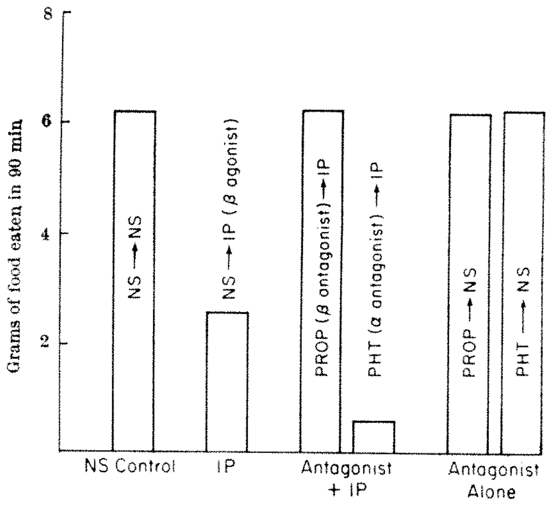

Fig. 1. Effects of hypothalamic injection of the β-adrenergic agonist, isoproterenol (IP), and of adrenergic antagonists upon food intake in food-deprived rats. The β-adrenergic antagonist is propranolol (PROP), the α-adrenergic antagonist is phentolamine (PHT), and the control medium is normal saline (NS). All drugs were injected directly into the hypothalamus. The score is mean grams eaten during the first 90 min after injection. The following meaningful comparisons were statistically reliable, at $P < 0\cdot001$: bars 1 : 2, 1 : 4, 2 : 3, 2 : 4, 3 : 4, 4 : 6.

A total of twenty-five male albino Sprague–Dawley rats (320 g) were stereotaxically implanted with unilateral chronic cannulas under 'Nembutal' anaesthesia, using the procedure described by Slangen and Miller[4]. All placements were aimed at the perifornical region of the hypothalamus immediately posterior to the anterior hypothalamus.

The following drugs were used: (–)-Norepinephrine bitartrate, an α-agonist (0·02 μmoles), (–)-isoproterenol bitartrate, a β-agonist (0·15 μmoles), (–)-epinephrine bitartrate, a β and α-agonist (0·10 μmoles), propranolol hydrochloride, a β-antagonist (0·14 μmoles), and phentolamine hydrochloride, an α-antagonist (0·07 μmoles). All drugs, except phentolamine, were dissolved in normal saline. Phentolamine was dissolved in distilled water. All drugs were injected directly into the hypothalamus through the same cannula, and the volume of each injection was always 1 μl.

All rats were fed and tested on Purina lab chow pellets. The first test consisted of injecting these rats, while food and water-satiated, with norepinephrine. During the first 30 min after injection, their food intake was significantly ($P < 0\cdot001$) increased from 0·2 g after saline to 2·7 g after norepinephrine. This eating response evoked by a centrally injected α-agonist agrees with the results of previous investigations[3,4] and confirms our first prediction.

All subsequent tests were carried out on the rats when they had been deprived of food for 18 h. The test, given every 3 to 4 days, consisted of measuring the rats' food intake during the first 90 min after drug injection. The rats were divided into two groups, one to be tested with

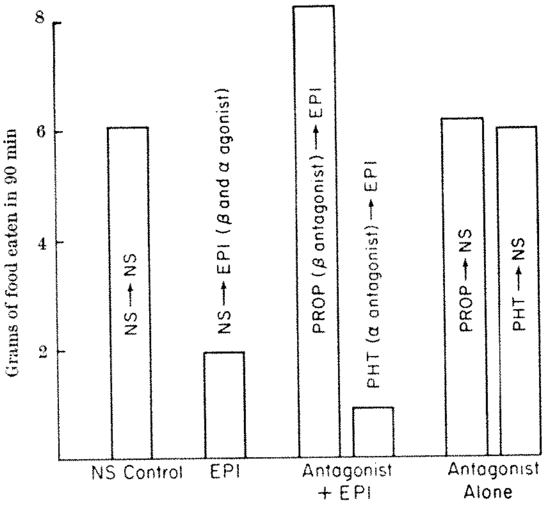

Fig. 2. Effects of hypthalamic injection of the β and α-adrenergic agonist, epinephrine (EPI), and of adrenergic antagonists upon food intake in food-deprived rats. The β-adrenergic antagonist is propranolol (PROP), the α-adrenergic antagonist is phentolamine (PHT), and the control medium is normal saline (NS). All drugs were injected directly into the hypothalamus. The score is mean grams eaten during the first 90 min after injection. The following meaningful comparisons were statistically reliable, at $P < 0{\cdot}001$: bars 1 : 2, 1 : 3, 1 : 4, 2 : 3, 2 : 4, 3 : 4, 3 : 5, 4 : 6.

isoproterenol, the potent β-agonist, and one with epinephrine, the potent β and α-agonist. Each rat in the two groups received six pairs of drug treatments, according to a Latin square sequence. The two injections of each pair were separated by 5 min. The six paired treatments for the two groups, which had either isoproterenol or epinephrine as their agonist, were: saline→saline, saline→agonist, propranolol→agonist, phentolamine→agonist, propranolol→saline, and phentolamine→saline.

The results of these experiments (Figs. 1 and 2) confirm all the predictions generated by the hypothesis that the regulation of hunger in the rat is mediated by hypothalamic α and β-adrenergic systems and that the receptors of the α-system elicit eating behaviour and the receptors of the β-system suppress eating behaviour. Unilateral hypothalamic injection of isoproterenol and epinephrine, two potent β-agonists, had a strong and almost immediate anorexic effect, which confirms our second prediction. These two drugs reliably suppressed food intake by 60 per cent and 70 per cent, respectively. These results, illustrated in the first two bars in Figs. 1 and 2, contrast clearly with the eating response elicited in satiated rats by hypothalamic injection of norepinephrine (the first prediction), and they provide strong evidence for the existence of hypothalamic β-adrenergic "satiety" receptors.

The third and fourth bars in Figs. 1 and 2 show the pronounced effects which the two centrally injected adrenergic antagonists have on anorexia elicited by hypothalamic β-adrenergic stimulation. These results confirm the third, fourth and fifth predictions in every respect. Propranolol, the β-antagonist, but not phentolamine, the α-antagonist, completely blocked the anorexia elicited by isoproterenol and epinephrine (third prediction), a finding which clearly demonstrates that such drug-induced anorexia is mediated by central β-adrenergic receptors. In the epinephrine-injected rats, propranolol induced a significant reversal of the effect of epinephrine on hunger, converting its anorexic effect into a strong hunger-stimulating effect (the fourth prediction). This epinephrine "reversal", which reveals the antagonistic relationship between hypothalamic α and β-receptors, caused these already hungry rats to eat 35 per cent more than they did when injected just with normal saline, or with propranolol and normal saline (bars 1, 3 and 5 in Fig. 2). The anorexic effect of isoproterenol, although completely blocked by propranolol, was not reversed in the direction of hunger-stimulation, presumably because its α-receptor activity is too weak to elicit an enhanced eating response (bars 1 and 3 in Fig. 1). Hypothalamic pretreatment with phentolamine, the α-antagonist, reliably potentiated both isoproterenol and epinephrine-induced anorexia, which confirms the last prediction (bars 2 and 4 in Figs. 1 and 2).

Neither the α nor the β-antagonists had any effect of their own on food intake at the relatively low doses used in this experiment (bars 5 and 6 in Figs. 1 and 2). Preliminary results from this laboratory, however, show that, at somewhat higher doses, phentolamine and propranolol suppress and enhance food intake, respectively. These findings lend further support to the hypothesis that the physiologically active central hunger-regulating mechanism in the rat consists, at least in part, of two adrenergic systems, α and β, which interact antagonistically.

Finally, the concepts and techniques presented in this study were used to investigate the well known amphetamine-induced anorexic effect, which is not expected on the basis of the norepinephrine-releasing properties of this drug. These investigations have yielded very interesting data which strongly suggest that the suppressing effect of amphetamine on food intake is mediated by hypothalamic β-adrenergic "satiety" receptors.

These findings confirm the hypothesis that hunger in the rat is regulated by a hypothalamic α and β-adrenergic mechanism. These central α and β-receptors interact in an antagonistic manner, with the α "hunger" receptors eliciting eating behaviour and the β "satiety" receptors suppressing eating behaviour. These results demonstrate that hunger and satiation can now be neurochemically differentiated in the hypothalamus of the rat.

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Integration and Analysis of Movement Information by the Visual System of Flies

INTERNEURONES capable of discriminating motion have been found in the medulla, lobula and central brain of such insects as *Calliphora phaenicia* and *Musca domestica*[1-3]. These interneurones discharge selectively in response to simple motion in one direction. I report here the demonstration of units that respond to complex motion.

Unitary discharges were recorded from the medulla, lobula and other brain regions of *Boettcherisca peregrina* through glass microelectrodes with tips less than 0·3 μm in diameter. Stimuli were given by moving a light spot, 2 mm in diameter, projected on to a white screen 10 cm in front of the fly. The light from a microscope lamp with source intensity 5 V was conducted to the screen by a light guide 2 mm in diameter and 1 m in length.

Almost all units which respond to a moving spot were found also to discharge in response to steady illumination within their receptive fields, but the discharge frequency was not as great as in the response to the moving spot. Two examples of units that responded to complicated motion are shown in Figs. 1 and 2. The unit illustrated in Fig. 1 responded to motions in the left half of the visual field, the centre of which was on a line extended from the axis of the head, as described by Bishop *et al.*[2]. The only motions which elicited spikes were: downwards in the left half of the visual field, from right to left in the upper left quadrant, and from left to right in the lower left quadrant. In other words, this unit discharged when the spot was moved counterclockwise in the left half of the visual field. The other unit (Fig. 2) responded when the spot was moved counterclockwise in the complete visual field. Several examples are shown in Fig. 3. Neurones with the response pattern illustrated on the right in *A* discharged in response to motions in all directions. Units shown on the left in *A* responded mainly to motions of two directions, upward and left to right, but also discharged with lower spike frequency in response to motions in the opposite directions. These two kinds of neurone were found in the optic medulla region. The visual field was relatively wide (visual angle of about 180°) for these units. Neurones which could discriminate complicated motion, such as are illustrated in *B*, were found in the optic lobula region. The size of the visual field for these units was relatively moderate (visual angle of about 90°) or narrow (30°–40°). The visual field of the unit shown in the middle of *B* was limited to the upper half only. The motion pattern illustrated in *C* was extremely analytical and also integrative. This unit responded to motion in two directions, upward and right to left, in the narrow central area of the visual field (visual angle of about 30°), while it discharged in response to motions in the opposite directions, downward and left to right, in the area surrounding the central visual field. This unit was found in the corpora ventralia of the brain. I also found the following units: a unit that inhibited the spike discharges corresponding to a fixed spot, or the spontaneous ones, when the spot moved in one direction; an "alerting" unit that discharged only when the spot started to move and did not elicit spikes if successively stimulated in the same way; and a unit that responded to clockwise movements of the spot.

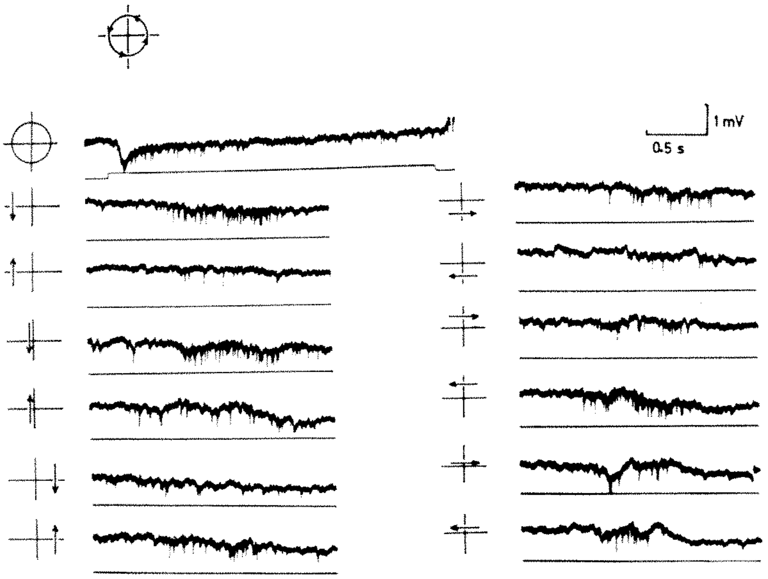

Fig. 2. Responses of a directionally selective unit to a moving spot. This unit discharged when the spot moved counterclockwise around the centre of the visual field (see inset), regardless of the distance from the centre.

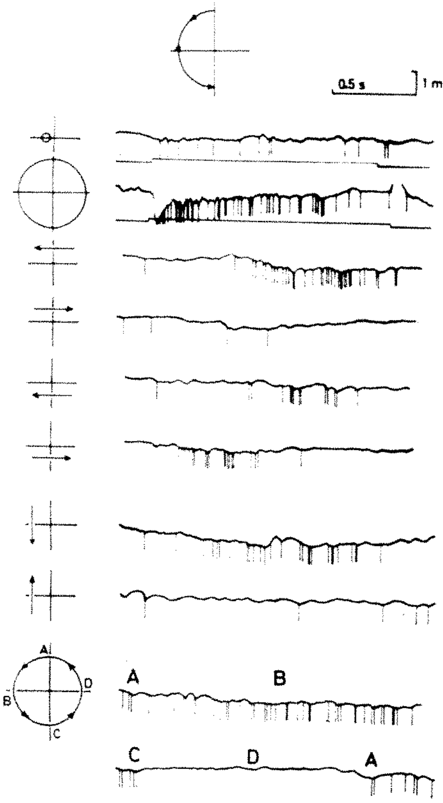

Fig. 1. Responses of a directionally selective unit to a moving spot. The geometrical centre of the visual field, which is on a line extended from the axis of the head, is indicated by a cross. Arrows show the direction of movement of the spot. As shown in cases where the spot moves horizontally, discharges are scarcely elicited at all when the spot moves through the right half of the visual field. In other words, this unit discharges when the spot moves counterclockwise in the left half of the visual field, as indicated in the bottom (*A* to *C*) and top inset. Discharges were also elicited by steady illumination in one spot (top record) or in all visual fields (second record).

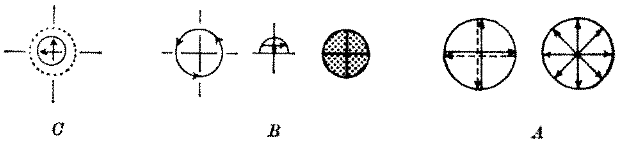

Fig. 3. Examples of the patterns of motion discrimination. Units found in the peripheral region (optic medulla) responded non-selectively to motions in various directions (right of *A*), or were merely capable of gross discrimination of motion (left of *A*). Arrows with a broken line indicate that the discharge frequency in response to the movement of the spot in the direction shown is less than that denoted by arrows with the solid line. In the lobula region (*B*), the motion pattern is more complicated or integrative than in the medulla region. The shaded circle indicates inhibition of discharges by illumination (right of *B*). The unit denoted in the middle of *B* responded to motions in two directions in only the upper half of the visual field. The unit shown in *C* was found in the corpora ventralia of the brain. It responded to movement of the spot in two directions within the narrow central visual field and to motions in the opposite directions in the surrounding visual field (indicated by dotted circle).

In general, the following tendencies seem to be present: with transmission of the visual information from the peripheral neurones to the central structure, (1) the direction of motion responded to is restricted, (2) the discrimination of motion becomes strict, as shown by the reduction or elimination of spikes in response to motions in the opposite direction, and (3) directions of motion are combined in a complex way. Further, in a few neurones of the central brain the discrimination of motion becomes so strict that (4) the direction of the motion which elicited spikes was opposite for the central and surrounding visual fields, and (5) the discriminable speed of movement of the spot was limited. These findings seem to provide interesting data about the perception of moving objects and its biological meaning, especially with respect to the perception of circulatory motion: for example, in relation to the sense of equilibrium when the body rotates in flight.

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Determination of a Behavioural Transfer Function by Frequency Analysis

TRANSFER functions are used by engineers as a means of describing, in a single equation, the relationship between the input and output of a system. Frequency analysis is a classic method of determining transfer functions in physical systems, and has also been used for quasi-behavioural systems, such as those controlling optokinetic responses[1] and the pupillary light reflex[2]. In the behavioural context, a transfer function describes the stimulus–response relationship in all possible stimulus conditions. It also makes it possible to compare biological and physical systems directly. In other words, it offers an explanation of behaviour that is independent of the "hardware" involved. In this preliminary report, frequency analysis is applied to behaviour, with the eventual aim of accounting for motivational phenomena rigorously and quantitatively.

The water intake of many species is positively correlated with ambient temperature, and in some species temperature-induced water loss may be responsible for increased drinking[3]. The evidence, however, suggests that pigeons drink in direct response to ambient temperature changes, in the absence of dehydration (refs. 4, 5 and our unpublished results). Our study deals with the determination of a transfer function relating thirst motivated behaviour to ambient temperature.

Three Barbary doves (*Streptopelia risoria*) were trained to obtain 0·1 cm³ water rewards by pecking at an illuminated key in a Skinner box, which was installed inside a climatic cabinet. Rewards were delivered on a random interval schedule, with a mean interval of 2 min (VI 2 min). The animals were trained for ten weeks, until they responded at a constant rate for a 3 h period after 48 h without water.

The animals were tested every two days, and were water deprived during the intervening periods. Tests were conducted alternately at a constant temperature of 15° C and at a temperature fluctuating sinusoidally between 5° C and 25° C. Tests were run at frequencies of 0·40, 0·50, 0·66, 1·00, 1·33, 2·00 and 4·00 cycles/h. The accuracy of the sinusoidal temperature function was ±0·25° C. Pecks delivered and rewards obtained were recorded on an event recorder throughout the whole of each 3 h test period, but the records for the first and last 30 min were discarded to avoid possible effects of initial transients and satiation respectively.

The results showed that the mean response rate was the same at all frequencies and distribution of rewards was not changed as the response rate fluctuated. The results of two sample test sessions at different frequencies are shown in Fig. 1. Preliminary examination of the results indicated that the frequency response was similar to that of a first-order system. On this basis, the appropriate transfer function is given by

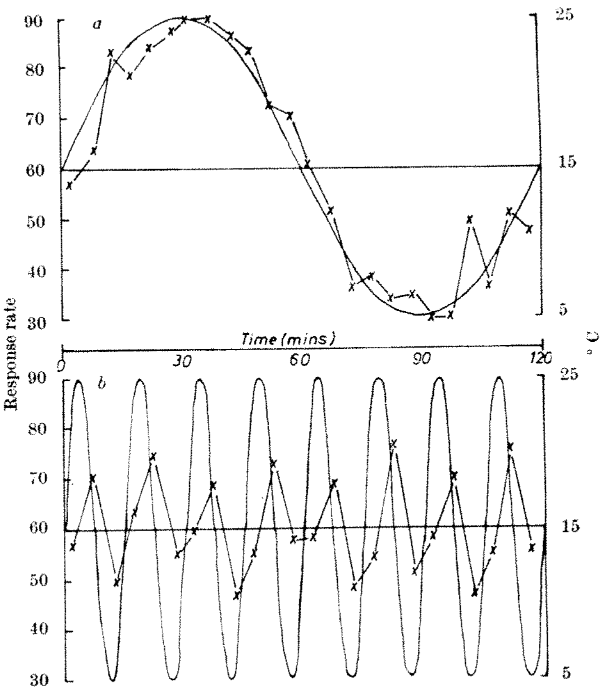

Fig. 1. Sample results at temperature frequencies of (*a*) 0·5 cycles/h and (*b*) 4 cycles/h. Response rate in pecks/5 min is indicated by crosses, ambient temperature by the continuous curve.

$$H(i\omega)=\frac{k}{i\omega T+1}$$

where k is the gain and T the time-constant of the system. The phase angle between input and output is given by

$$\varphi=-\text{arc tan }\omega T\triangleq\angle H(i\omega)$$

The results provide a series of values of φ for corresponding values of ω. For example, at a frequency of 1 cycle/h the observed phase-lag is 31·2°.

$$\varphi=-\text{arc tan }\omega T=-\text{arc tan }2\pi T=-31{\cdot}2$$

From this equation, $T=0{\cdot}6056/2\pi T$ h$=5{\cdot}78$ min. On average, the value of T was found to be equal to 5·57 min, and this value forms the basis of the phase plot illustrated in Fig. 2*a*.

The expected values of the amplitude ratio AR as a function of frequency can now be calculated.

$$AR=|H(i\omega)|=\frac{k}{(1+(\omega T)^2)^{\frac{1}{2}}}$$

$$\log AR=\log_{10}k-\text{c}\tfrac{1}{2}(\log_{10}(1+(\omega T)^2))$$

An AR value of 1 can be defined when two conditions are satisfied: (1) that the average response rate is the same at the lowest observed frequency as at infinite frequency; and (2) that the same empirical response amplitude is found at two different low frequencies. The ratio between these and the temperature amplitude is then arbitrarily defined as unity. In practice, both these conditions were satisfied, and the procedure was used to construct the predicted curve in Fig. 2*b*, namely

$$\log_{10}AR=-\tfrac{1}{2}(\log_{10}(1+(\omega T)^2))$$

Comparison with the observed AR, calculated on the same basis, indicates good correspondence with the expected values (Fig. 2*b*), and to this extent the hypothesis of a first-order system is confirmed.

The results indicate that appetitive drinking behaviour can occur as a rapid response to changing ambient temperature. The time-constant of the response, 5·57

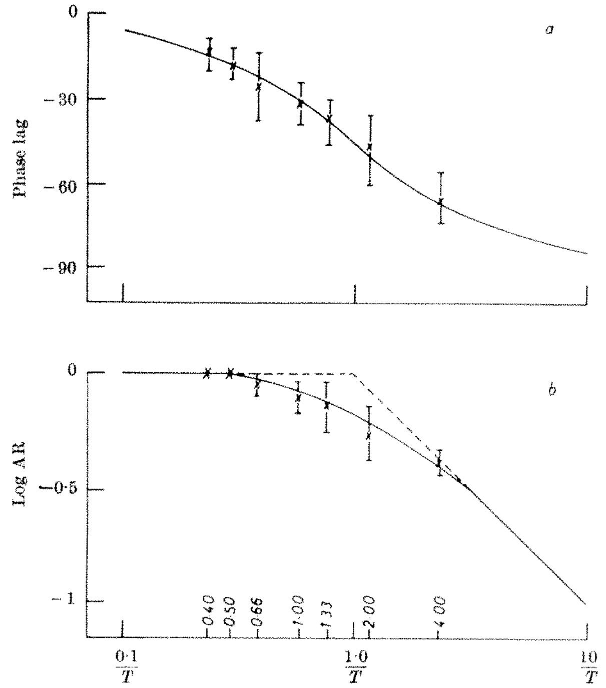

Fig. 2. Bode plot of mean response rate as a function of temperature frequency. *a*, Observed phase lag matched to that of a first-order system (continuous curve); *b*, observed compared with expected (continuous curve) amplitude ratio. Vertical lines indicate the range.

min, can be compared with a time-constant of about 2 min for the hypothalamic temperature response to a step change in ambient temperature in the 48 h water deprived Barbary dove (our unpublished results). It is possible, though we cannot yet be sure, that the drinking response is mediated by hypothalamic temperature changes, and that the increased time-constant results from the heat production caused by the work involved in the operant response.

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Pheromones and their Effect on Aggression in Mice

Odours from the urine of male mice modify the sexual state of females, and this capacity has been attributed to the presence of one or more pheromones[1-4]. Male urine also possesses a pheromone which increases aggression in other male mice[5,6], but it has not been identified and its source has not been located. We wanted to know whether the output of aggression pheromone by the male is related to its agonistic behaviour, and whether the production of pheromone is androgen dependent and therefore reduced by castration. We also wanted to investigate the activity of female urine, because the precise properties of a female which permit close socio-sexual contact with a male are not clear.

Male albino mice, one hundred days old, were used. Twenty-five test mice which had been isolated for 10 days were each fought against twenty-five treated, non-aggressive, castrate opponents, and their aggressive behaviour observed for 3 min.

Pooled urine samples, taken fresh from donor mice which had been placed in metabolic cages, were applied to the coats of the castrate opponent mice, immediately before introducing them into the test cage. Urine donor categories were as follows: (1) six aggressive and (2) six non-aggressive males selected from twenty isolated males which had been fought against each other by the round-robin method[7]; (3) six bilaterally castrated males which had been operated on at 90 days of age; (4) six normal females. A fifth group of opponent mice was treated with water.

The aggressiveness of the test mouse against its regular castrate opponent was assessed by the latency period to the first attack, the number of bites and the total time spent attacking the opponent mouse. These tests were run on alternate days to blank test sessions, when the control castrate opponents received no treatment. The order of testing any group of treated mice was randomized.

Table 1. MEAN SCORES OF TEST ISOLATE MICE, ON FOUR MEASURES OF AGGRESSION, WHEN THEIR OPPONENTS WERE GIVEN DIFFERENT URINE/WATER TREATMENTS

	Source of urine applied to standard castrate opponents				
	Agg.	Sub.	Cast.	Water	Female
Latency measure					
Mean test session score with treatment	87·2	93·0	109·0	108·6	149·5
Mean change in score following treatment	−30·7*	−11·6	+2·5	11·1	+55·9*
Accumulated attack score					
Mean test session score with treatment	18·1	19·6	14·8	13·4	6·7
Mean change in score following treatment	+3·6†	+1·6	−1·04	−1·8	−10·9*
Bites score					
Mean test session score with treatment	10·0	9·9	8·4	6·4	3·3
Mean change in score following treatment	+2·4†	+1·0	−0·4	−0·7	−5·8†
No. of fights out of 25					
Number with treatment	20	16	15	13	12
Change in number following treatment	+4	+1	−4	−4	−9

Probability values (one-tailed) derived from analysis by the Wilcoxon sign test for correlated samples.

* $P < 0·025$; † $P < 0·05$.
Agg. = aggressive; sub. = submissive; cast. = castrate.

The results in Table 1 show that less of the aggression-promoting pheromone is produced in submissive than in dominant males, and none at all in castrate males. A Friedman two-way analysis of variance revealed significant overall differences between five groups (latency measure $X^2 = 12·7$, $P < 0·025$; bites score $X^2 = 11·7$, $P < 0·025$; accumulated attacking score $X^2 = 12·7$, $P = 0·025$). Our results are consistent with the findings that the male pheromones which accelerate the oestrous cycle[2] and block ovo-implantation of recently inseminated female mice[3] are also eliminated by castration. Because the male pheromone is androgen dependent, the fact that the effectiveness of submissive male urine is intermediate between that of aggressive and that of castrate males may be attributed to differences in androgen output. In this connexion we know that isolation increases both androgen output and aggression in male mice[8] and we have also found (unpublished results of N. W. N.) that dominant males, selected by the round-robin test, have significantly larger ventral prostates than their submissive fellows. It would seem therefore that aggression and aggression-inducing pheromone production are stimulated by a common factor.

Females produce a pheromone that has an oestrus-suppressing effect on other females[4]. Our results demonstrate that female urine contains a factor that inhibits male mouse aggressiveness. Without this control, presumably, sexual contact would be less successful. In sexually dimorphic species, size, colour and shape might provide the female cues for recognition by the male. We suggest that, in mice, urinary pheromones may serve this function.

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Protean Displays as Inducers of Conflict

SINCE Charles Darwin's great work of 1859, various facets of the biological armament race between predator and prey have been studied in depth, as in the work of Blest[1], Cott[2], Darwin[3] and De Ruiter[4]. These studies describe and analyse regular, predictable structure/behaviour complexes, such as camouflage, mimicry and various fleeing mechanisms.

Many animals, however, increase their efficiency as protagonists by using types of behaviours which are irregular and thereby defeat detailed prediction. We have surveyed the occurrence of such protean behaviours and defined them as behaviours which are sufficiently unsystematic in appearance to prevent a reactor predicting in detail the position and/or actions of the actor (ref. 5 and our unpublished work). The simplest and most widespread example is the single erratic display of a fleeing animal, characterized by unpredictable turns[5]. Many species, including man, will perform this display when attacked. Roeder[6] has photographed it in noctuid moths and provided evidence of its survival value[7].

The basic problem in understanding protean phenomena has been to explain how natural selection could operate in reducing the predictability of behaviour. The increase of irregularity and variety seems in part to be the opposite of evolutionary ritualization which enhances the signal value of movements or postures. In this communication we suggest that experimental psychology provides the theoretical basis which can explain the survival value of protean displays. We propose that a protean display arouses mutually incompatible tendencies in the reactor—in other words, it induces conflict. A result of conflict is lengthened reaction time[8], which must reduce a predator's chances of capturing prey.

Protean displays induce conflict in several ways. First, the predator may be presented with simultaneous stimuli, each requiring a different response, as when an attacked group of animals scatters, producing conflicting directing responses. Effects on the predator's mood may also be involved, as in the aggression of a convulsing *Peromyscus*[9]. Conflict may be similarly induced by a very rapid sequence of stimuli, each arousing a response which cannot be completed before the next stimulus has appeared and set up competing processes. Examples include a rapid single erratic display, and any distraction display involving more than one actor.

Second, the erratic nature of a protean display defeats anticipation by the predator. The more frequently stimulus events are associated in the input to an organism, the greater will be the tendency for the central correlates of one to activate the central correlates of the other[10]. Thus one stimulus may evoke preparatory responses which will allow an optimal reaction to a second stimulus. This is useful when the organism is dealing with systematically ordered stimulus systems, but protean displays seem to be highly unsystematic. Thus many preparatory responses will be wasted, or may lead to lowered efficiency by conflicting with the behavioural processes required by the new situation. This is likely when a predator is following an animal performing a single erratic display, and also when there is a change in role or position within a displaying group, as in mobbing. In its extreme form this kind of conflict appears as "surprise", which in human subjects is associated with an arrest of movements[11].

Third, when a predator is faced with the possibility that any one of several alternative stimuli is about to occur, each requiring a different response, the predator is in a state of uncertainty. Such situations involve conflict by the anticipatory arousal of incompatible responses[12]. In the single erratic display this would mean that the predator's responses with regard to direction of movement would be incompatible. Again, variations in the display given by prey on different occasions will induce conflict even in the experienced predator. If a predator has learned that its prey has various avoidance behaviours, anticipatory conflict automatically increases its reaction time.

Fourth, novel stimuli will resemble to an approximately equal extent several other familiar stimulus patterns, and by generalization will arouse certain conflicting responses appropriate to all of them[13]. The avoidance of novel objects is greatest when they have some familiar features[14]. Novel stimuli also promote uncertainty, for there is no way of knowing what will follow them. Those aspects of a protean display which are novel to the reactor therefore induce conflict. An example is the repeated flashes of bright colour during the convulsive erratic skipping of many butterflies and moths when disturbed (our unpublished work). Even a single flash of colour can delay a predator's attack[1].

The degree of conflict between competing response tendencies is determined by their nearness to equality in strength, their absolute strengths and their number[14,15]. An increase in any of these factors lengthens reaction time.

Finally, game theory[16] indicates that when two protagonists have a fairly equal chance of success, as in the "which hand is it in?" game, the best pattern is no pattern. Unpredictability is increased to the maximum possible for that particular system, the structure of which becomes protean. In the game mentioned this sets a fundamental limit of 50 per cent to predictability; this cannot be overcome by learning in the protagonist. Unlike systematic escape displays, protean displays are therefore highly resistant to learned countermeasures by the predator.

Protean behaviour embodies irregularities and stimulus variety as basic principles which increase the reactor's reaction time. This could mediate the selective advantage by which an escape display becomes protean; and the widespread occurrence of protean properties in escape behaviour suggests that they are a major factor in the biological armament race.

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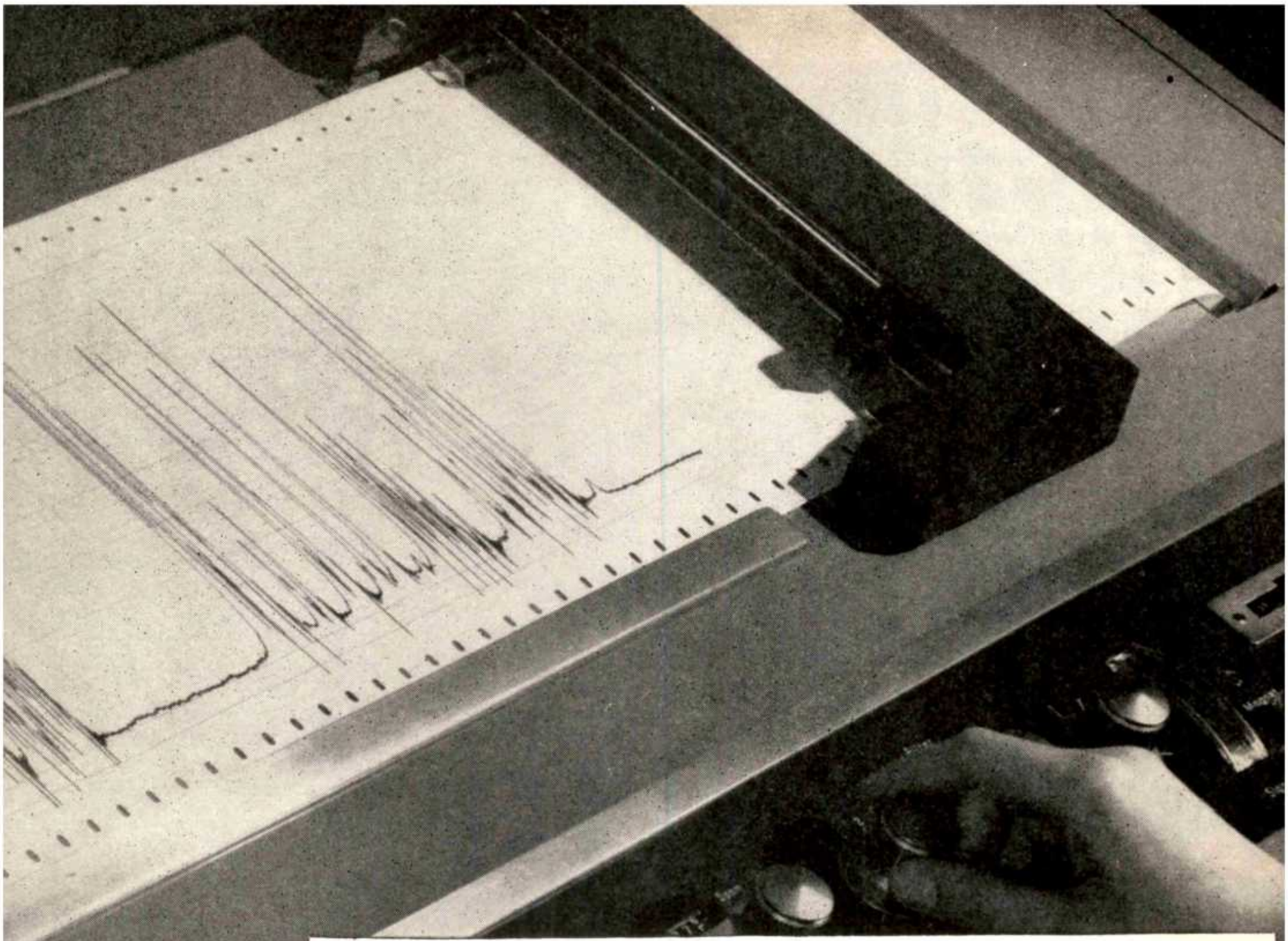

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Nitrous Acid Effect on Cleavage and Development in Fertilized Frog Eggs

NITROUS acid causes deamination of primary amino groups attached to the ring structure of nucleic acid bases; the products have been demonstrated to cause mutations in viruses[1,2], bacteria[3], moulds[4,5] and yeasts[6]. Apart from a study demonstrating chromosomal fragmentation in monocotyledon root tips treated with nitrous acid[7], no report of HNO_2 having an effect on higher organisms has been found.

I treated fertilized frog eggs with HNO_2 and observed cleavage and development up to the feeding larval stage. The eggs, which still have jelly membranes intact, respond quickly to the externally applied HNO_2. This is advantageous, because many chemical inhibitors of development are only effective following internal injection in frog eggs[8]. HNO_2 was found to affect cleavage and development and the result is dependent on concentration and time. Furthermore, depending on the experimental conditions, the reaction of the eggs is a delayed one, for although the chemical may be removed from the eggs at one stage, the eggs do not stop developing until much later. HNO_2 may therefore have damaged nucleic acid before its expression during development.

Artificial fertilized *Rana pipiens* eggs were treated with HNO_2 solutions ($NaNO_2$-sodium acetate, *p*H 5·0) or control solutions (see Table 1). The eggs were subjected to varying times of treatment at room temperature (23°–25° C), and then rinsed in 10 per cent Steinberg's salt solution[9] and placed in about 150 ml. of this solution. Early cleavage and development up to Shumway stage 25 (ref. 9)—the feeding larval stage—were observed.

The effects of nitrous acid are enhanced as the $NaNO_2$ concentration is increased (Table 1). Controls indicate that this cannot be ascribed to the effects of *p*H, buffer alone, or sodium nitrite (legend to Table 1), but if the concentration of sodium acetate is increased at constant concentration of $NaNO_2$, cleavage and development are affected at the higher buffer concentration.

Table 1. EFFECT OF NITROUS ACID TREATMENT ON FERTILIZED FROG EGGS

$NaNO_2$ (M)	Acetate *p*H 5 (M)	Calculated HNO_2 (M)	Cleavage	Development to stage 25
0·05	0·01	0·001	0	0
0·01	0·01	0·0002	+	0
0·05	0·005	0·001	0	0
0·01	0·005	0·0002	+ +	+
0·05	0·0025	0·001	+	0
0·03	0·0025	0·0006	+ +	0
0·02	0·0025	0·0004	+ +	+
0·01	0·0025	0·0002	+ +	+ +
Control solutions			+ +	+ +

The number of eggs are indicated by: none (0), like controls (+ +), or less than controls (+). Eggs were added to solutions 1½–2 h after fertilization and treated for 3–4 h (16–32 cell stage). In each of the groups, 35–45 eggs were treated in 8 ml. of solution. Control solutions were 0·0125 M sodium acetate buffers, *p*H 5·0 and 7·0; 0·05 M $NaNO_2$–0·0125 M sodium acetate buffer, *p*H 7·0; and 10 per cent Steinberg's, *p*H 7·4. Because HNO_2 is unstable, the $NaNO_2$ solutions were made up fresh in glass distilled water adjusted to *p*H 7·1–7·3 with a weak NaOH solution. The *p*H of the HNO_2 solutions during treatment of the eggs remained within 0·1–0·2 *p*H units.

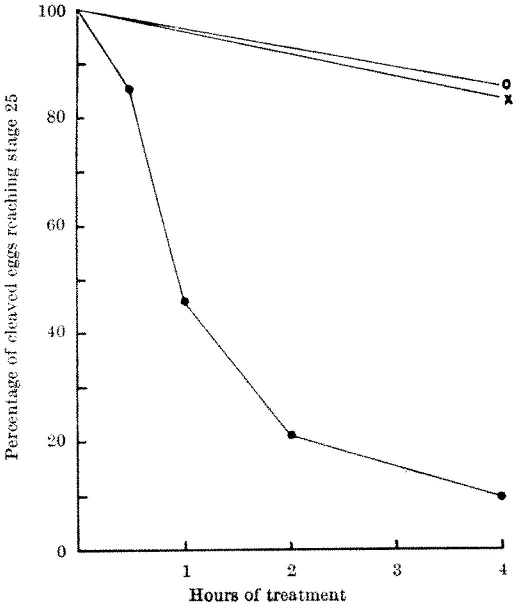

Fig. 1. Number of cleaved eggs reaching stage 25 after treatment with 0·02 M $NaNO_2$–0·0025 M sodium acetate buffer, *p*H 5·0 (●); 0·0025 M sodium acetate buffer, *p*H 5·0 (○); or 10 per cent Steinberg's solution, *p*H 7·4 (×). Eggs were added to solutions at first cleavage. 40–50 eggs were used per group.

As the length of exposure to nitrous acid is increased, fewer embryos reach a higher developmental stage (Fig. 1). A 4 h treatment with 0·02 M $NaNO_2$ caused a ten-fold decrease in the number of cleaved eggs to reach stage 25. Fig. 2*a* also demonstrates that the nitrous acid effect depends on length of treatment and concentration. Furthermore, the eggs may be treated with the chemical at one stage—early cleavage—yet development is not affected until later stages—blastula, gastrula or tail bud. A 20 h treatment in 0·01 M $NaNO_2$ resulted in a halt in development of 90 per cent or more of the eggs at the late blastula stage (stages 8–9). When these treated eggs were transferred to 10 per cent Steinberg's solution there was no further development. This is interesting because mRNA synthesis is thought to begin at an increased rate as a result of gene activation at this time in development[10].

The time in development at which HNO_2 is added to the eggs is important (Fig. 2*b*). When 0·05 M $NaNO_2$ is added to eggs 90 min after fertilization (shortly before first cleavage), all eggs stop developing immediately: yet 50 per cent of the eggs treated within minutes after fertilization for approximately the same length of time develop as far as stage 25. I have also observed that the eggs react more strongly to HNO_2 at first cleavage than shortly after fertilization: this effect might reflect easier penetration of the chemical at first cleavage.

In general, the jelly coats and egg membranes did not manifest external changes after brief exposure to solutions of HNO_2. The nature of the jelly coats does seem to affect the rapidity of the response to HNO_2 (Fig. 2*c*): eggs with jelly layers developed to stage 25 after a 10 min treatment with 0·05 M $NaNO_2$, whereas most de-jellied eggs only developed to tail bud (stage 17). The HNO_2 presumably enters eggs containing jelly layers within 30 min because some of the eggs with jelly coats treated for 30 min stopped developing after late cleavage.

Preliminary experiments indicate that in certain conditions HNO_2 may affect development of only one cell type, namely the germ cells. For example, when eggs were treated for 4 h with 0·02 M $NaNO_2$ only 10 per cent of the eggs reached stage 25 (Fig. 1). These embryos are normal in appearance except that the germ cells are significantly reduced in number. Ultraviolet treatment of amphibian eggs selectively damages nucleic acid material

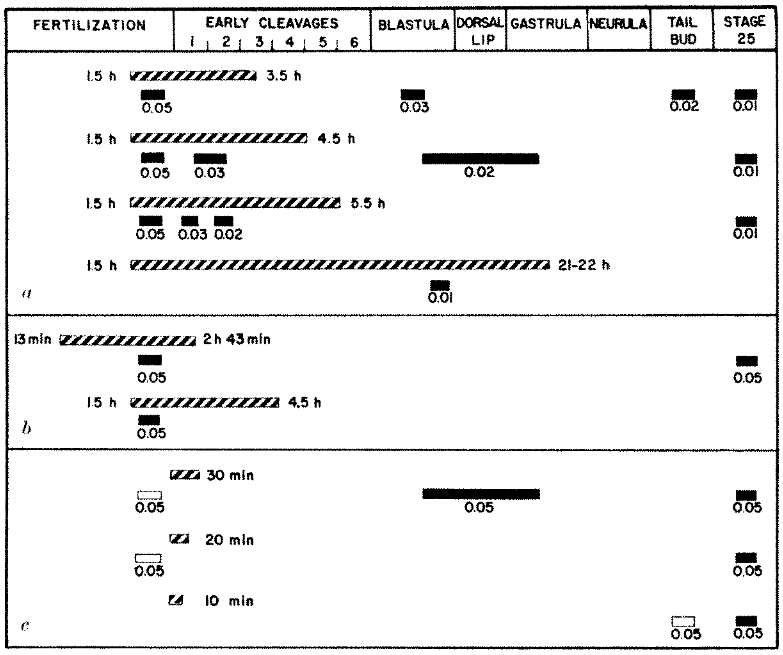

Fig. 2. Hatched areas refer to length of nitrous acid treatment. In *a* and *b* the times before and after these areas are the number of hours after fertilization when eggs were added and removed. In *c* the times refer to the duration of treatment. The black areas indicate the stage to which most ($\geqslant 40$ per cent) of the eggs developed at a particular molarity of $NaNO_2$. 0·005 M sodium acetate buffer, *p*H 5·0, was used with 0·05 M $NaNO_2$; 0·0025 M sodium acetate buffer, *p*H 5·0, was used with the other $NaNO_2$ molarities. *a*, Varying length of treatment in and concentration of nitrous acid. 40–50 eggs per group. *b*, Varying time when eggs are added to HNO_2. 40–50 eggs per group. *c*, Effect of HNO_2 on eggs with jelly coats (black areas) and de-jellied eggs (open areas). 20 eggs per group.

found in the egg cytoplasm (germ plasm) and results in normal embryos which lack germ cells[11]. Because HNO_2 affects nucleic acid, it is conceivable that one of its mechanisms of action is on the germ plasm.

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Isolation of a Protein responsible for Aggregation of Avian Embryonic Cells

CELL adhesion, as a mechanism of morphogenesis in higher organisms, has been explained by two contrasting hypotheses: first, by adhesive macromolecules[1], and, second, by a lyophobic colloid mechanism[2]. Lilien and Moscona[3] have reaggregated retina cells with a supernatant preparation taken from a culture of retina cells without, however, investigating the nature of the active compound. Electron microscopy investigations[2] have suggested that there are gaps of 100–200 Å between the plasmalemmas of living chicken embryonic cells and of many tissues of a variety of species, but were unable to find any intercellular material. We have examined the reaggregation of avian cells to identify the nature of the molecules causing reaggregation if such were found to exist.

After surgical removal, the retinas of 10 day old chick embryos were treated with 0·25 per cent trypsin (Commonwealth Serum Laboratory, Melbourne) for 10 min, washed twice in Hanks balanced salt solution and centrifuged. Then 6×10^7 of the disaggregated cells were resuspended in 8 ml. Eagle's basal medium (Grand Island Biological Co., New York) with 15 per cent foetal or young calf serum (Commonwealth Serum Laboratory, Melbourne). These were then incubated at 37° C, *p*H 7·2, for 48 h in 2 oz medical bottles. After incubation, the medium was discarded and the cell monolayers were washed three times with Hanks solution and the medium was replaced by 4 ml. Eagle's basal medium (EBM) for another 24 h. This second medium was removed and the monolayers were washed with Dulbecco "A" salt solution. To each bottle was then added 3 ml. of 0·05 M ethylene-diamine-tetraacetate, adjusted to *p*H 7·3 with 1·0 M NaOH, and after gentle pipetting to disperse the tissue, the bottles were incubated for 10 min at 37° C. The suspension was agitated, then removed and centrifuged immediately at 9,500 r.p.m. for 30 min. It was shown that the EDTA treatment did not reduce the viability of the disaggregated cells nor cause any leakage of cell components. The supernatant was freeze dried, and the dry matter was dissolved in 5 ml. of glass-distilled water and passed through a column (2·5 × 30 cm) of 'Sephadex *G*-25'. The column was eluted at 4° C with glass-distilled water and that fraction of the eluate corresponding to the excluded molecules was freeze-dried. The material was dissolved in Tyrode solution, and the protein content was determined by the method of Lowry *et al.*[4].

The biological activity of the isolated material was determined by using explanted retinas of 10 day old chick embryo disaggregated with freshly prepared trypsin (0·25 per cent) for 10 min at 37° C. Cells were counted with a haemocytometer and 5×10^6 cells placed in 25 ml. Erlenmeyer flasks containing the material dissolved in 3 ml. Tyrode solution. Each cell culture was also provided with 10 µg deoxyribonuclease[5] I (bovine pancrease; Sigma). The medium was adjusted to *p*H 7·2; the bottles were closed with a rubber stopper and placed in a gyratory shaker operated at 72 r.p.m. at 37° C. The effect of the medium on cell aggregation is shown in Fig. 1. The degree of aggregation is measured in terms of the mean log diameter of aggregates.

Although there was no significant aggregation in Tyrode solution (*A*), numerous compact aggregates were formed both in Eagle's basal medium plus serum (*B*) and with the material we prepared and refer to as retina active substance (RAS) in Tyrode solution (*C*). The effect of RAS concentration on the aggregation of retina cells is presented in Fig. 2.

The association between RAS concentration and mean log diameter of aggregates indicates that even 3 µg/ml. will induce a marked cell aggregation, while the peak value is between 30–100 µg/ml. Inhibition of cell aggregation was found at 200 µg/ml. After this concentration-dependent aggregation of retina cells had been found, we investigated the nature of the binding compound by using trypsin (lyophilized Trypsin 2 × crystallized, Worthington Biochemical Corporation, New Jersey).

RAS was treated with trypsin, at trypsin : RAS ratios of 1 : 1 to 1 : 50 at 37° C in a gyratory shaker for periods of 2, 6 or 12 h. Soyabean trypsin inhibitor (grade 1, Seravac Laboratories) was then added at twice the trypsin concentration (because of its relative impurity), and incubation continued for another hour. Finally, 5×10^6 retina cells were added after adjusting with Tyrode to 3 ml. and they were incubated for a further 24 h. The results show that trypsin treatment of 2 h did not greatly affect the activity, but the 6 and 12 h trypsin treatment eliminated the aggregating ability of the RAS. These results will be published elsewhere in detail. Control experiments indicated that neither trypsin nor soyabean trypsin inhibitor alone caused any cell reaggregation.

Recent examination of embryonic chick cell re-aggregates by electron microscopy after lanthanum staining[6] revealed intercellular material composed of fibrils. The results of our experiments suggest that protein is responsible for the adhesion of retina cells during the process of reaggregation and we suggest that reaggregation is caused by protein molecules in the intercellular spaces. In view of the extractability of the RAS with EDTA it is further suggested that the cell plasmalemmas are bridged to RAS by divalent cations.

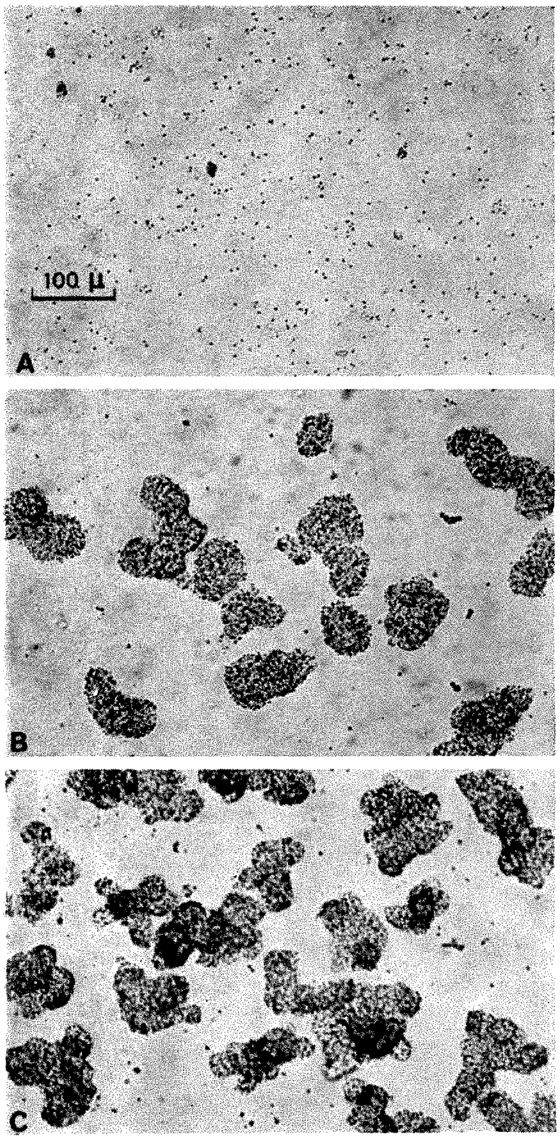

Fig. 1. The reaggregation of retina cells in: *A*, Tyrode solution; *B*, Eagle's basal medium plus 15 per cent serum; *C*, retina active substance in Tyrode salt solution (× 50).

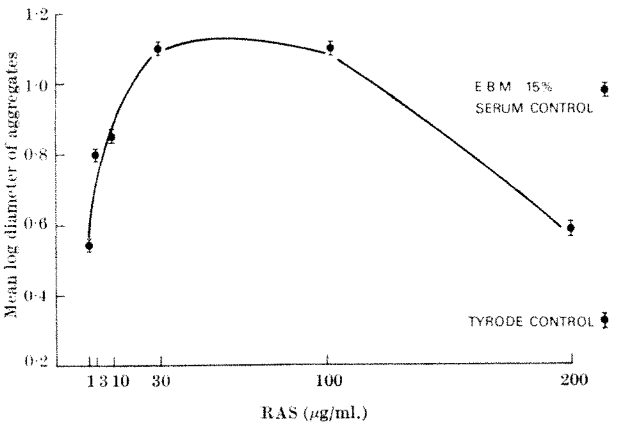

Fig. 2. Relationship between the concentration (μg/ml.) of retina active substance (RAS) and the mean log diameters of aggregates (bars denote mean and standard error).

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Evidence against Transmission of Scrapie by Animal House Fomites

Accidental contamination is an abiding anxiety for all who work with scrapie agent and the possibility of false positive results is well recognized[1]. Few, however, have sought to evaluate this risk experimentally. In the light of the report by Morris *et al.*[2], we have examined the role of close physical contact and animal house fomites (sawdust, droppings, scrapers, and so on) in the establishment of the disease in normal animals.

Swiss mice—originally derived from the Moredun stock—were kept in totally separate "normal" and scrapie animal houses. All experiments were carried out in the latter and mouse adapted scrapie agent[3] was used. Animals were examined carefully twice a week, and reported on daily by animal house attendants. All findings were checked histologically (by Cajal staining of astrocytes) and by passage into further Swiss mice.

In contact experiments, normal mice were kept for more than two years in an animal house normally reserved for scrapie infected animals. They usually became old and ruffled. Ordinary scrapers were used in cleaning their cages: no particular regime was instituted, but scrapers were dipped in 'Tego' disinfectant in the usual way between cages. In a second experiment, lasting 22 months, groups of six Swiss mice were inoculated intracerebrally with 0·05 ml. of a 10 per cent suspension of scrapie brain ("10^{-1}") and put either immediately or after 14 days into groups of twenty-four C57 black mice. The Swiss mice were removed as soon as their condition was severe, care being taken to prevent them dying in the cage.

In breeding-contact experiments, animals were bred from one or two scrapie infected parents. Usually two litters, occasionally three, were obtained. Sometimes they were suckled by the original mother (if normal), but most were fostered to normal animals. After being weaned, litters were kept in the scrapie animal house for 20 months. Other litters, from normal animals, were reared from birth in the scrapie animal house.

In experiments with cage refuse the faeces and contaminated sawdust from cages of mice which had received intracerebral injections with scrapie 4 weeks previously, were transferred into the cages of normal recipient mice twice a week. This was continued until the scrapie animals were far advanced but discontinued if an animal was found dead or had been cannibalized in the scrapie cage. The experiment (with successive batches of scrapie mice) has proceeded for 20 months.

Other experiments concerned inadequately autoclaved apparatus. First, a McCartney bottle in which a whole scrapie mouse brain had been stored in about 15 ml. of 10 per cent formalin was emptied and, without rinsing, a normal brain was put into it together with 15 ml. fresh formalin. After 4 weeks this normal brain was homogenized and inoculated into mice (two separate experiments carried out). Second, an MSE macerator used for making a suspension of scrapie brain was autoclaved at 1·4 atm for 30 min once (usually this is done twice). A suspension of normal mouse brain was then prepared and inoculated intracerebrally into normal mice. Third, a suspension of 10^{-1} scrapie mouse brain was autoclaved for 30 min at 0·5 atm and then inoculated intracerebrally into normal mice. Similar experiments were carried out after autoclaving at 1·2 and 2·0 atm. In all cases titrations to 10^{-6} were carried out. The original material was similarly titred.

At no time did any mouse develop scrapie through contact with infected animals. This is true of animals which shared a cage with the latter as well as of those in separate cages within the scrapie animal house. Progeny of scrapie parents were never observed to develop the disease during 22 months, with the exception of one animal from a litter of six which was bled from the tail a few weeks after birth to test for lactate dehydrogenase level in the blood.

Animals encountering soiled litter from scrapie animals took on an aged appearance—ruffled hair, slightly hunched, slow movements, rather easily pushed over—much sooner than normal. After about a year they looked twice their age. No scrapie was detectable in any animal, however, though the astrocytes were hypertrophied (especially in the vicinity of small blood vessels) in the manner common to old animals.

The use of a contaminated bottle did not result in scrapie in the recipient mice during 2 years of observation; nor did the use of an imperfectly autoclaved macerator result in the disease. When scrapie brain suspension was autoclaved at 0·5 atm for 30 min, five of six animals injected with 10^{-1} developed scrapie, the incubation periods ranging from 8–12 months. The one remaining animal is normal. At 10^{-2}, one of six animals developed the disease after 11 months. After autoclaving at 1·2 atm, inoculation at 10^{-1} produced scrapie in one of four animals after 11 months. No scrapie developed after 2·0 atm autoclaving. Control material (not autoclaved) contained scrapie down to 10^{-6}.

In a second experiment, scrapie material (one-tenth suspension) was autoclaved for 1·3 atm at 30 min. It produced scrapie in one out of four mice after 13 months' incubation. The others are well after two years. Passage of the brain of the positive mouse produced full blown disease in 6 months. When the scrapie material was autoclaved a second time in the same conditions, it produced scrapie in four of five mice, 13, 14, 15 and 18 months after intracerebral inoculation. Again further passage of these animals resulted in the disease in 6 months. Experiments are in progress with the same material subjected to a third autoclaving process.

It is a common fear that scrapie may contaminate normal mice or experimental mice when attempts are made to establish other putative slow infections. Indeed, Morris *et al.*[2] reported the occurrence of seven cases of scrapie among two hundred normal mice which had been housed in the same room as affected mice, the disease making its appearance among the normals 18 or 19 months later. Morris *et al.* thought it likely "that virus might have been carried from cage to cage by forceps, scattered bedding, or unwashed hands, mixed water bottles or cages, or even insufficiently sterilized cages in the long period of over one year of daily handling and feeding". The experiments described here, in which healthy animals were exposed to contaminated litter, do not support this suggestion. The observations of Morris *et al.* are of the greatest importance in assessing transmission attempts so it is important to consider them critically. Unfortunately not all their "scrapie" mice were fully examined histologically, and even more important—for histological diagnosis of scrapie in old mice can be difficult or even impossible—passage was not carried out. Some doubt must accordingly attach to their results.

Fitzsimmons and Pattison[4] have reported unsuccessful attempts to transmit scrapie to sheep, goats, mice or rats by nematode parasites. Pattison[5] found that scrapie did not occur in scrapie susceptible (Cheviot) sheep or goats kept indoors in contact with a succession of affected animals; nor was he able to transmit the disease with faeces or saliva—findings which fit in well with our observations. Only when scrapie and normal animals inflicted injury in fighting did cross-infection occur —apparently from ingestion of infected fragments of tissue. Chelle[6], on the other hand, reported a case of scrapie in a goat which had been in continuous contact from birth with scrapie affected sheep. More recently, Brotherston *et al.*[7] found scrapie in ten out of seventeen goats kept for a long time in contact with sheep suffering from natural scrapie, but contact infection (in a different institute) did not occur when sheep with experimental scrapie were used as a source of infection.. Indeed, as long ago as 1954, Sigurdsson[8] presented evidence that the agent of rida (the Icelandic form of scrapie) might survive outside the host on farms where it had been prevalent and invoked an intermediate host or vector. Zlotnik[9] reported contact spread in four out of forty-two mice in 15–16 months, though the normal animals might have licked some of the inoculum leaking from the injection site. But in our study no spread was seen following either immediate contact or contact delayed for 14 days after inoculation. Contact within a cage has also been reported to result in spread[10], and Dickinson and Stamp (unpublished work cited in ref. 7) believe that lateral contagion may sometimes occur in the field.

Only Morris *et al.*[2] have reported the development of scrapie among normal animals housed with scrapie infected animals, and our work—specially designed to test for it in the worst possible conditions of contamination —has not revealed it. Animals exposed to contaminated cage litter seem to "age" more rapidly than usual but no case of scrapie has been proven histologically or by passage. We conclude that the dangers (in this particular animal house) are very small. Transmission experiments nevertheless have always been carried out in a separate animal house.

There are, however, two sources of error which may well affect long-term experiments. The possibility cannot be entirely excluded that an occasional escaped animal is replaced (instead of being killed). A wrongly labelled bottle may also be a source of error. Occasionally the disease may be inadvertently transmitted by ear-punching normal animals with an instrument used for scrapie animals[11] and infection might be carried over during withdrawal of blood by tail snipping with contaminated scissors. Handling of normal material with contaminated instruments cannot be ruled out, though direct experiment suggests that carelessness would have to be gross. On the other hand, very low scrapie titres may lead to greatly prolonged incubation periods. Recently a mouse injected with erythrocytes taken from an animal inoculated with scrapie intracerebrally 2 h previously, developed the disease after 17 months. Incubation periods beyond 6 or 7 months were found by Pattison and Jones[12], who reported that scrapie occasionally resulted from inoculation of preparations from non-scrapie brain or tumour material.

Thus we conclude that when large numbers of animals become ill with scrapie within 7 or 8 months of inoculation of a material there can be little danger in accepting the findings as valid[13]. Very long incubation may be associated with low titre scrapie and such conditions have yielded some of the most interesting findings[12]. Further contamination experiments—especially with imperfectly sterilized instruments and glassware—are therefore necessary.

We thank Mr A. Keith and Mrs F. E. Bird for their care of the animals.

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Gigantopithecus and the Mountain Gorilla

PILBEAM[1] has recently described the peculiarities of the extinct pongid genus *Gigantopithecus*, and commented on the striking parallelisms between its functional morphology and that of both *Theropithecus* (following Jolly[2]) and the early Hominidae. I hope to show here that a similar functional complex has evolved in a living pongid, the rare mountain gorilla (*Gorilla gorilla beringei*).

Mountain gorillas live in the Virunga Volcanoes of the Great Lakes region of Central Africa, and on nearby Mt Kahuzi. To the west, in the Congo lowlands and in the less elevated ranges of Itombwe and Tshiaberimu, lives the eastern lowland gorilla (*G. g. graueri*); while 700 miles to the west of this subspecies occurs the western lowland gorilla (*G. g. gorilla*), living in the region between Nigeria and the lower Congo.

Table 1

	G. g. gorilla (Cameroun) ♂ Skull measurements (mm)	Sample size	*G. g. graueri* (Utu) ♂ Skull measurements (mm)	Sample size	*G. g. beringei* ♂ Skull measurements (mm)	Sample size
Cranial length	198·5	112	186·5	12	200·2	13
Maxillary toothrow	66·3	112	71·3	12	73·5	13
Palate length	117·1	112	126·3	12	134·8	13
Ascending ramus ht.	127·1	98	124·1	11	133·7	
Bigonial breadth	128·4	98	128·1	11	144·6	14
Mandibular length	204·0	98	205·6	11	206·9	14

The differences between the three subspecies concern skull characters, limb proportions, coloration and so on, and are described in a forthcoming paper[3]. The mountain gorilla, while not of much greater size than the other forms, has much larger teeth, and correspondingly more developed jaw musculature, than the western gorilla; the eastern lowland form is intermediate. A good idea of the differences can be gained from the average values[4] for male skulls (measurements in mm) in Table 1. It can be seen that *beringei*, the mountain gorilla, has larger teeth, a longer palate, higher ascending ramus and more widely flaring jaw angles than a lowland gorilla of the same

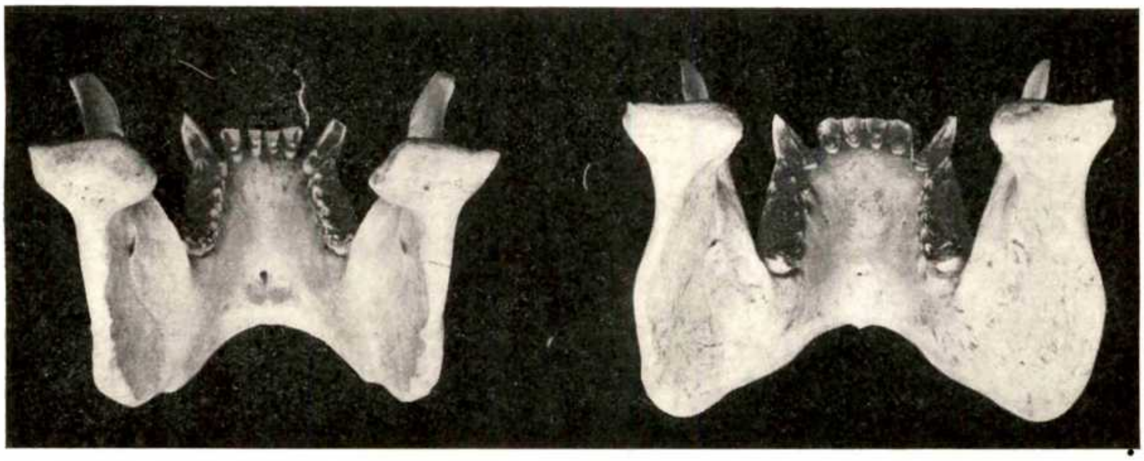

Fig. 1. Mandibles of *G. g. gorilla* (left) and *G. g. beringei* (right). Note the higher ascending ramus and more flared jaw angles of the latter. (Photo by Uno Samuelsson, Naturhistoriska Riksmuseet, Stockholm.)

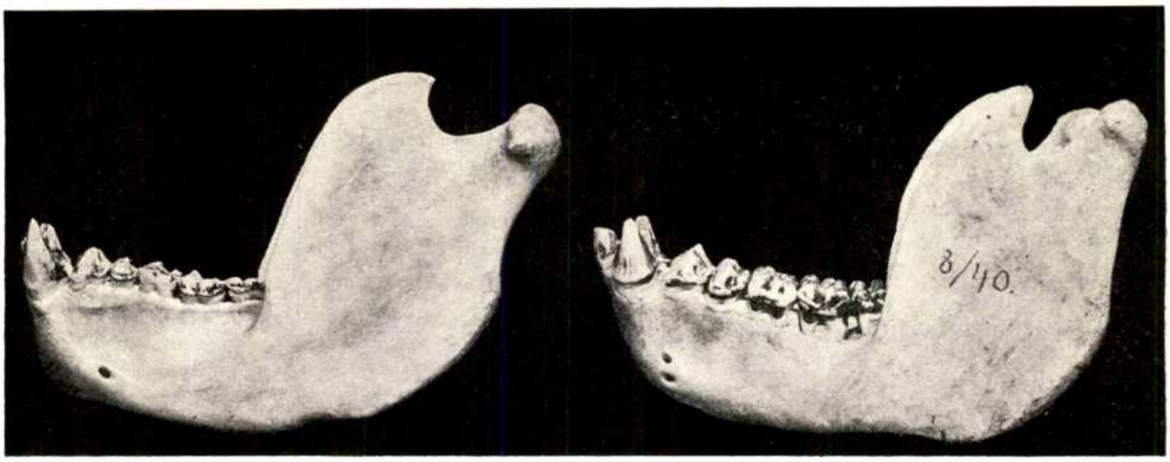

Fig. 2. Mandibles of *G. g. gorilla* (left) and *G. g. beringei* (right). Note the larger cheek teeth with higher crowns, and the higher, more muscularly moulded ascending ramus of the latter. (Photo by Uno Samuelsson, Naturhistoriska Riksmuseet, Stockholm.)

size. The eastern lowland gorilla has the large teeth and long toothrow of the mountain gorilla and a fairly long palate, but in other respects resembles its western lowland relative. The differences between highland and lowland gorillas thus to some extent recall those between *Gigantopithecus* and its dryopithecine forbears. The mandibular length of the three races also does not differ markedly, so that the long toothrow of the mountain gorilla is contained in a space no greater than that for the smaller teeth of the western gorilla. In connexion with this, Vogel[5] has observed that M_3 in *beringei* more often disappears behind the ascending ramus than in other gorillas.

Groves and Napier[6] have calculated indices to quantify the relative sizes of incisor row, canine (mesiodistal) length and molar row. Table 2 shows these indices in the mandibles of male gorillas (eastern lowland gorillas excluded). Thus the anterior teeth of the mountain gorilla, incisors and canines equally, are reduced compared with the cheekteeth just as in *Gigantopithecus*.

Table 2

	G. g. gorilla[6]	*G. g. beringei*[6]
Incisors to molars	30·3– 32·2	26·4– 30·6
Canine to molars	31·2– 37·3	29·6– 33·0
Canine to incisors	111·1–120·6	111·4–125·0

Dr Ulrich Welsch has drawn my attention to other features of the teeth of *G. g. beringei*: notably the tendency to develop extra cusps on both upper and lower teeth (5th and 6th cusps on the upper, 6th cusps on the lower), these being commonest on M^3_3, followed by M^2_2, and rare in *G. g. gorilla*. Multicuspidate molars are listed by Pilbeam[1] as distinctive of *Gigantopithecus*. The mountain gorilla tends also to have a more rounded, less sectorial form of P_3 than lowland forms—corresponding to the reduced anterior dentition. The jawbone of *beringei* is thicker, more massive and more muscularly moulded than that of *graueri* or *gorilla*[5].

According to Schaller[7], the mountain gorilla eats roots, bark and similar hard-wearing, bulky items, while in the Utu region of the "eastern lowlands", leaves and fruit are more favoured items; pith and the growing parts of the stem are eaten in both areas. The western lowland gorilla apparently eats similar foods to the eastern lowland race, and both consume a large amount of cultivated plants. The data therefore support Pilbeam's interpretation of the function of the *Gigantopithecus* morphology. The differences I have described between mountain and lowland gorillas can be said to be an intensification of those between the gorilla as a whole and the chimpanzee. The ecological and especially dietary data from the two species support this view.

Pandey and Sastri[8] have recently described a new dryopithecine species from the Siwaliks which they name *Sivapithecus lewisi*. The description indicates a large form, apparently foreshadowing certain *Gigantopithecus* traits: M_1 is high-crowned and measures $13{\cdot}9 \times 12{\cdot}1$ mm, by far the largest and marginally the narrowest dryopithecine teeth known from the Siwaliks; the canine is slightly reduced: P_3 is less sectorial, and as broad as it is long. The mandibular corpus is extremely deep and rather thick. Because posterior molars are lacking, no direct comparison with *Dryopithecus giganteus* is possible, but it may be that "*S.*" *lewisi* in fact represents this latter species, considered by Pilbeam to be ancestral to *Gigantopithecus*.

The mountain gorilla is a living member of the Hominoidea, in which direct ecological comparison with conspecifics is possible. Such comparison may help to elaborate the functional meaning of the "*Theropithecus* complex" which Pilbeam has usefully applied to *Gigantopithecus*, and which has recently[2] been applied by its describer, Jolly, to the early Hominidae; it should be clearly understood, however, that the concentration on small, tough objects—the ultimate elaboration of the *T*-complex—cannot be made to apply to *Gigantopithecus* or the gorilla, and is characteristic of man and *Theropithecus* alone.

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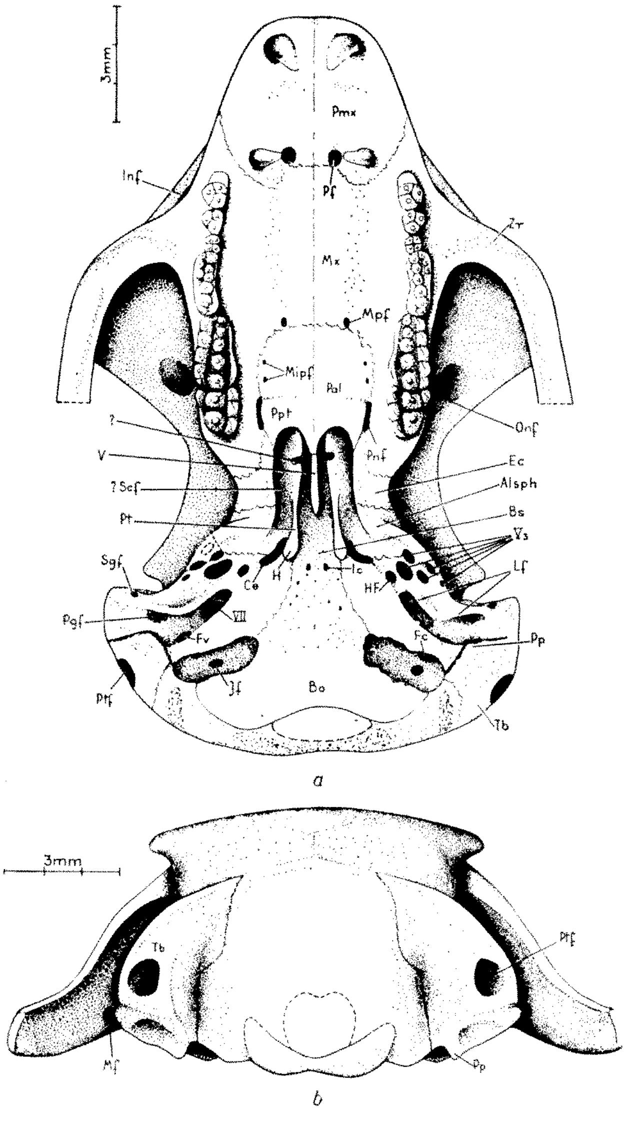
Unknown Structures in Multituberculate Skull

UP until now, no complete multituberculate skull has been known. Although skulls of two Palaeocene genera, *Ptilodus* Gidley and *Taeniolabis* Cope, are nearly complete, they both lack the lateral wall of the braincase and the occipital region[1]. Large collections of Cretaceous multituberculates recovered recently in North America through application of underwater screening techniques have yielded isolated teeth and skull fragments[2–4]. A collection of Cretaceous multituberculates has also been recovered from Bayn Dzak (Shabarakh Usu) in the Gobi Desert in

Mongolia by the Polish-Mongolia palaeontological expeditions[5]. The Gobi collection is smaller than that from North America, but it is more complete, having some entire skulls associated with fragments of the postcranial skeletons[6,7]. The age of the Bayn Dzak multituberculates is estimated as Coniacian or Santonian[6].

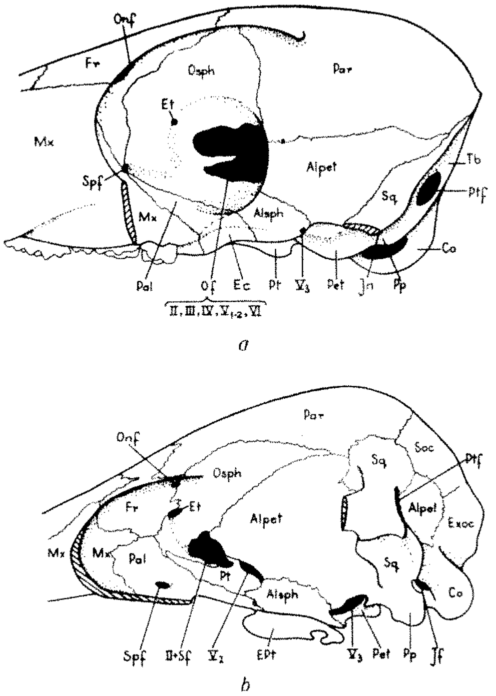
The type specimen of *Kamptobaatar kuczynskii* Kielan-Jaworowska[6], assigned to the Taeniolabididae, is the most complete multituberculate skull yet found. From a study of this skull, I have been able to recognize the following structures, previously unknown in Multituberculata. The pterygoids (Fig. 1*a*) do not form the lateral walls of the choanae, but are situated in the middle of either side of the choanae. Thus the choanae are divided by the vomer and the pterygoid bones into four channels. The inner channel on either side served as an air channel, while the lateral one, ending in a cul-de-sac, served as an area for muscle attachment. Lateral to the choanae there is an ectopterygoid bone. This consists of a rectangular posterior part and triangular anterior process, inserted between the palatine and maxilla. The ectopterygoid tapers at the palatonasal foramen[8]; posteriorly, it contacts the alisphenoid. There seems to be a suture between the alisphenoid and the petrosal. In the antero-lateral corner of the promontorium, presumably along the suture between the alisphenoid and the petrosal, there is a comparatively large opening which may be a remnant of cavum epiptericum. The foramen for V_3 is divided into five foramina, which probably lay along the line of fusion between the alisphenoid and the petrosal or partly within the petrosal. The alisphenoid forms a "wing", which surrounds the foramina for V_3 antero-laterally, protruding downwards over the basicranial region. The division of the foramen for V_3 into five foramina is characteristic only of *Kamptobaatar*. In other Bayn Dzak multituberculates, as in *Ptilodus*[1], the foramen for V_3 is divided into two foramina. The remaining foramina in the basicranial region in *Kamptobaatar* are more or less of the same pattern as in *Ptilodus*.



a

b

Fig. 1. *Kamptobaatar kuczynskii* Kielan-Jaworowska, reconstruction of the skull, *a*, occlusal view; *b*, occipital view (the anterior part of the snout seen in this view is not shown in the figure). Alpet, anterior lamina of the petrosal; Alsph, alisphenoid; Bo, basioccipital; Bs, basisphenoid; Ec, ectopterygoid; Ept, Echnidna pterygoid; Et, ethmoid foramen; Exoc, exoccipital; Fc, fenetra cochleae; **Fr**, frontal; Fv, fenestra vestibuli; H, hamulus; HF, hiatus canalis Fallopii; Ic, internal carotid foramen; Inf, infraorbital foramen; Jf, jugular foramen; Jn, jugular notch; Lf, lateral flange; Mipf, minor palatine foramen; Mpf, major palatine foramen; Mx, maxilla; Mf, maxillary foramen; Of, orbital fissure; Onf, orbitonasal foramen; Osph, orbitosphenoid; Pal, palatine; Par, parietal; Pet, petrosal; Pf, palatine fissure; Pgf, postglenoid foramen; Pmx, premaxilla; Pnf, palatonasal foramen; Pp. paroccipital process; Ppt, postpalatine torus; Pt, pterygoid; Ptf, post-temporal fossa; Scf, scaphoid fossa; Sf, sphenorbital fissure; Sgf, supraglenoid foramen; Spf, sphenopalatine foramen; Sq, squamosal; Tb, tabular; Zr, zygomatic ridge; II, III, IV, V_1, V_2, V_3, VI, VII, foramina for respective cranial nerves.



a

b

Fig. 2. Diagrammatic drawings of the lateral aspects of the braincase in *Kamptobaatar* (*a*) and in *Ornithorhynchus* (*b*). Not to scale. Abbreviations as in Fig. 1.

The lacrimal bone and lacrimal foramen are lacking in all Bayn Dzak multituberculates. The sphenopalatine foramen and the ethmoid foramen are well preserved in *Kamptobaatar*. The orbitosphenoid has a very large exposure in the orbit. It seems probable that a part of the interorbital wall was membraneous in life, and it is presumed that the large orbital fissure (see Fig. 2) passed the following nerves: II, III, IV, V_{1-2} and VI. The exposure of the perpendicular part of the palatine and of the ectopterygoid and alisphenoid in the lateral wall of the braincase is recognized tentatively. The alisphenoid is probably reduced to a comparatively small, ventral element, which does not contribute very much to the structure of the lateral wall of the braincase. Most of the lateral wall of the braincase is occupied by the anterior lamina of the petrosal, which is very extensive and irregular in shape. The squamosal has little exposure on the lateral wall of the skull, and the reconstruction of its shape in *Ptilodus* given by Simpson[1] does not seem to be very probable.

In the occipital plate (Fig. 1*b*), there is an extensive tabular which occupies the entire region between the occipital and lambdoidal crest. In the lower part of the tabular there is an unusually large post-temporal fossa. The triangular paroccipital process (in the reptilian sense) is also characteristic of the occipital region of *Kamptobaatar*.

The structures I have described show that the skull of Cretaceous multituberculates is characterized by a juxtaposition of primitive (therapsid) and advanced (mammalian) characters. The general pattern of the multituberculate braincase, in contradistinction to the opinion of Simpson[1], is essentially the same as in the monotremes (Fig. 2). On the other hand, the studied ear region and the triradiate structure of the choanal region strongly resemble those in triconodonts[9] and docodonts (*Morganucodon*)[10]. This new information on the skull structure in multituberculates confirms the opinion of Kermack[11], that the Monotremata, Multituberculata, Triconodonta and Docodonta form one subclass Prototheria, equivalent to the Theria. The Multituberculata are, however, more allied to the Monotremata than they are to the Docodonta and Triconodonta. It seems that there is no reason to place the Multituberculata in the subclass of their own, Allotheria[12], and the theory of the polyphyletic origin of mammals[13] cannot be maintained.

I thank Dr K. A. Kermack, Zoology Department, University College, London, for discussion and for placing at my disposal the undescribed specimen of *Morganucodon* from China for an examination, and Dr L. P. Tatarinov, Palaeontological Institute, USSR Academy of Sciences, Moscow, for criticism and comments.

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Corophium curvispinum (Amphipoda) recorded again in the British Isles

Corophium curvispinum Sars. (Amphipoda: Corophidae) is a tubicolous crustacean which is spreading through the freshwaters of Europe, but has only been recorded twice before in the British Isles—once in the River Avon at Tewkesbury in 1935[1] (thirteen specimens), and at Stourport on Severn in 1962 (personal communication from R. W. Ingle) (sixteen specimens). In 1960, *Corophium* could not be found again at Tewkesbury[2].

Corophium has now been found in the Grand Union Canal in Leicestershire between Newton Bottom Lock (SP.631966) half a mile west of Newton Harcourt church, and at bridge 76 (SP.654941) half a mile north-east of Fleckney church. This represents a canal distance of approximately three miles. The collections were made between October 28, 1969, and February 11, 1970.

The collections have so far been concentrated on the *Fontinalis*, algae, silt and sponge debris on the brickwork of locks and bridges. The abundance and distribution of *Corophium* seem to be variable. Twenty or thirty specimens were collected quite easily at Newton Middle Lock (Spinney Lock), and at bridge 76 a hundred specimens could be picked out in a few minutes. Other collections yielded one or two specimens or none at all.

Now that *Corophium* is known to occur in the canal, a fuller survey is being made. There is also the possibility that a second species, *Corophium spongicolum*, may be found because sponges, on which it lives, are common in the locks and under bridges. Originally, *Corophium curvispinum* in freshwater was referred to as a variety 'devium'[3]. The latest opinion[4] is that two species, *C. curvispinum* and *spongicolum*, occur together in freshwater. It is difficult to understand how this animal has been overlooked until now, in view of the frequent collections that have been made over the years in the canal.

The discovery of *Corophium* in the canal was due to the recognition by a student, Miss Marion J. Gray, of a single specimen of an unusual crustacean, in a collection made by a field class from the School of Biological Sciences, University of Leicester.

I thank Dr R. W. Ingle of the British Museum (Natural History), who confirmed the identification and provided me with the latest information on the systematics of *Corophium*.

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Ethylene and the Respiration Climacteric

MANY theories have been proposed to explain the nature of the climacteric rise in respiration seen in most ripening fruits. They include changes in tissue organization which lead to increased metabolism[1], or effects of mitochondria which cause a loss of respiratory control[2]. The role of ethylene in the induction of ripening has now been confirmed but, in spite of much attention, the physiological nature and significance of the climacteric have remained elusive[3]. Although fruit tissues undergo many changes during ripening[4], much biochemical organization is retained; mitochondria do not lose their respiratory control[2] and protein synthesis continues up to the climacteric maximum[5-7]. Ripening has therefore been interpreted as a process requiring considerable cellular work, so that the climacteric is merely the respiratory summation of cellular energy requirements[3]. In many fruits, however, the energy requirements for the largely catabolic events of ripening must be very small. In the banana, for example, which shows a classical respiration climacteric, starch makes up as much as 20 per cent of the fresh weight before ripening[8]. During ripening, the starch is almost completely hydrolysed by phosphorylase to sugars (an exergonic reaction). Moreover, it has been shown that the respiratory climacteric can occur in the absence of protein synthesis[6].

The respiratory climacteric seems to be almost invariably associated with endogenous production of high concentrations of ethylene[3], the ethylene evolved after ripening begins being far greater than the amount that was required to start ripening in the same fruit. Some fruits ("non-climacteric" fruits) show no clear-cut respiratory climacteric and evolve very little ethylene during ripening. If such fruits are treated with ethylene (Fig. 1*a*), however, they show a marked respiratory rise, quite like a climacteric in time and magnitude. There seems there-

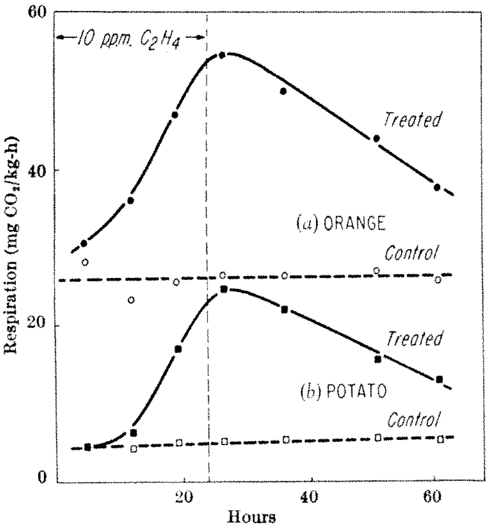

Fig. 1. Respiration at 20° C of (*a*) green mature sweet orange fruits (*Citrus sinensis*, seedling) and (*b*) mature potato tubers (*Solanum tuberosum*, cv. White Rose). The samples were held in a stream of air; ethylene was added to the treated lots during 24 h. Each point is an average of two (control) or four (treated) individual specimens.

fore to be a general association between the presence of high concentrations of ethylene and a respiration climacteric[9].

These observations support a different interpretation of the climacteric. The respiratory climacteric may be caused directly by ethylene itself at the large concentrations present in most ripening fruits, rather than by the energy requirements of other ripening phenomena. This in turn would suggest that the critical difference between the so-called "climacteric" and "non-climacteric" fruits is in the amount of endogenous ethylene produced during ripening. Although all fruits seem to be triggered to ripen by low concentrations of ethylene, only climacteric fruits show an autocatalytic production of ethylene, and consequently a respiration climacteric, as symptons of ripening.

Huelin and Barker[10] showed a small stimulation of the respiration of intact potato tubers on treatment with ethylene. We have re-examined this effect. At higher temperatures than they used, treatment of tubers with ethylene results in a very marked rise in respiration similar to that seen both in non-climacteric fruits treated with ethylene and in climacteric fruits (Fig. 1). At the respiratory peak, the respiration of treated tubers may be anywhere from five to ten times that observed in untreated tubers (Fig. 1*b*). Detailed examination of this phenomenon (unpublished work) shows other analogies to ripening fruits, particularly a marked increase in permeability. The condition of the potato, however, seems otherwise little affected by the treatment, and after a recovery period tubers will again respond to ethylene.

That treatment with ethylene of a tissue quite unlike a ripening fruit can cause respiratory effects similar to those long recognized in fruits supports the hypothesis that it may be ethylene itself that is responsible for the respiration climacteric in fruits. The potato may prove to be a useful model system for examining the role of ethylene in stimulating a respiratory climacteric in the absence of the array of other changes that occur in ripening fruits.

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DDT: its Action and Degradation in Bacterial Populations

A possible example of a bacterial population adapted to DDT has been reported by Henzell and Lancaster[1], who demonstrated a 50 per cent conversion of DDT in silage. In the study I report here, soil and water microflora were examined for DDT-accumulating, DDT-resistant, DDT-sensitive and DDT-degrading microbes.

DDT-accumulating organisms were detected by an autoradiographic technique[2-4]. Samples of water or soil suspension were streaked on to peptone agar with 0·8 p.p.m. ^{14}C-phenyl-DDT (specific activity 12·5 μCi/mg) in Petri dishes and incubated at 25° C. After colonies had developed, an X-ray film was placed on the agar, which had previously been covered with waterproof plastic foil (2 mg/cm^2). The film was exposed at 3° C in order to check microbial growth. After five days the film was developed and showed black spots (Fig. 1) corresponding to colonies that have accumulated ^{14}C-DDT, light spots corresponding to colonies that have not accumulated DDT and a grey background caused by radiation from the agar containing ^{14}C-DDT.

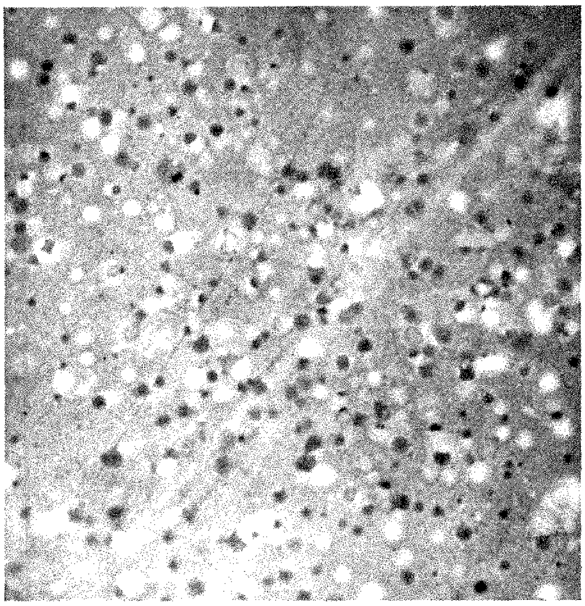

Fig. 1. Autoradiogram of peptone agar plate with 0·8 p.p.m. ^{14}C-DDT added. Black spots correspond to ^{14}C-accumulating colonies and white spots to non-accumulating colonies.

Among colonies grown from highly chlorinated Delft tap water, almost 1 per cent accumulated DDT. With polluted surface water the figures varied between 2 and 7 per cent, with garden soil samples between 60 and 70 per cent, and with nursery soil recently treated with a pesticide between 80 to 95 per cent. The percentage of DDT-accumulating bacteria was also found to be increased when other organochlorine compounds (endosulfan, lindane, simarin and captan) were applied as spray. In soil samples thought never to have been treated with any pesticide, 11 to 36 per cent DDT-accumulating bacteria were found.

The proportion of DDT-resistant bacteria in a population was estimated by a pour plate method: peptone agar containing various concentrations of DDT was mixed with a water or soil-suspension sample. The plates were incubated at 25° C. Daily colony counts showed striking differences in retardation and inhibition between two samples, of which one had been shown by autoradiography to contain 3 per cent DDT-accumulating bacteria, and the other 93 per cent (Table 1).

Table 1. DDT RESISTANCE IN TWO BACTERIAL POPULATIONS. SAMPLES ARE MIXED WITH PEPTONE AGAR CONTAINING VARIOUS CONCENTRATIONS OF DDT AND INCUBATED AT 25° C

Sample	Medium: peptone agar with DDT (p.p.m.)	Colony counts after 1–7 days expressed as percentages of the number counted after 7 days on plates with no DDT added: Days					
		1	2	3	4	5	7
Ditch water	0	7	38	78		92	100
3 per cent*	0·1	5	48	56		79	84
	1	6	36	74		89	91
	10	0	1	11		31	55
	25	0	0	0		0·5	0·5
Nursery soil	0	6	18	76	91	98	100
93 per cent*	25	0	2	12	15	16	16
	500	0	0·1	2	4	5	6
	1,000	0	0	0	0·1	0·4	0·5
	2,000	0	0	0	0	0	0

* Percentage of colonies in this population which showed blackening, as determined by the autoradiographic technique.

DDT-sensitive bacteria were detected by transferring peptone-grown colonies on to fresh plates with or without 1 p.p.m. DDT. Surprisingly, few colonies had failed to grow on these plates after three days. 5 p.p.m. DDT gradient agar plates, however, showed a definite inhibition zone. Pure cultures of the few strains which did not grow initially in the presence of 1 p.p.m. DDT could be induced to grow in two stages with 0·6 and 0·8 p.p.m. DDT.

Degradation of DDT by bacteria was investigated in pure cultures of strains selected from (*a*) strains which strongly accumulated DDT, detected by autoradiography, and (*b*) strains isolated from enrichment cultures in peptone water to which 2 per cent DDT had been added suspended in acetone. The bacteria were grown in peptone water with 1 p.p.m. ^{14}C-DDT on a rotary shaker at 25° C. After 20 h the cells were spun down, washed and extracted with methanol (once) and chloroform–methanol 1 : 1 (twice). The combined extract was put through a membrane filter, washed with 0·1 M KCl[5] and evaporated. The residue was dissolved in 5 ml. hexane and cleaned by extraction with dimethylformamide (DMF) (three times). Water was added to the combined DMF layer and DDT was left in the hexane[6]. The concentrated hexane was chromatographed on a silica gel plate with petroleum ether (40 : 60)–paraffin 4 : 1 (ref. 7). Radioactive spots were located by autoradiography and estimated with a Packard radiogram scanner. The membrane filter was also scanned for radioactivity. A recovery of 90–100 per cent was obtained from the filter and extracts combined.

One bacterial strain from group (*b*) accumulated almost 100 per cent of the radioactive material in the medium. By the methods described, 75 per cent was found in the extract and one third of this amount was chromatographically shown to have been converted into DDE. A slight amount of DDD was also present. The radioactive compounds left on the filter may be either strongly bound DDT or DDE, or unextractable degradation products.

The ubiquity of DDT—found even in Antarctic snow[8]—may explain the large proportion of DDT-resistant bacteria in all soil samples. On the other hand, its insolubility in water may explain the small proportion of DDT-resistant organisms in this ecosystem.

I thank Professor T. O. Wikén, director of the Laboratory of Microbiology, for his encouragement.

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Carbon Monoxide as a Basis for Primitive Life on Other Planets: a Comment

WOLFGANG[1] has made the interesting suggestion that photodissociation of carbon dioxide to $CO + O$ may provide the necessary ingredients for a chemotrophic primary production process conducted by living creatures on other planets, analogous to the primary production function of photosynthesis on the Earth. Wolfgang cites *Bacillus oligocarbophilus* as a terrestrial precedent for biological oxidation of carbon monoxide; *Hydrogenomonas carboxydovorans*[2] is a more recently described example.

Wolfgang made his suggestion partly to emphasize the point that speculation on these matters ought not to be prejudiced towards some variant of terrestrial photosynthesis. One ought also to avoid being influenced by the prevalence of aerobic metabolism in the world around us. Wolfgang's suggestion begs many questions about the stability of the O in the particular environment being considered; such questions could be largely avoided if CO oxidation were anaerobic. CO oxidation can be coupled biologically to sulphate reduction[3] and Yagi[4–6] has shown that it couples readily to the reduction of sulphite in extracts of sulphate-reducing bacteria. I have obtained extracts of *Desulfovibrio* which conducted a rapid oxidation of carbon monoxide with sodium dithionite (normally regarded as a very strong reductant) as the oxidizing agent. Anaerobic carbon monoxide oxidation, coupled to a step in the sulphur cycle, might be intellectually a somewhat more satisfying speculation than aerobic oxidation because of the circumstantial evidence now accumulating that ecosystems based on sulphureta are present-day representatives of some of the earliest terrestrial biological systems[7–9].

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Book Reviews

MAN OF MANY PARTS

Memories

By Julian Huxley. Pp. 296 + 22 plates. (Allen and Unwin: London, May 1970.) 63*s*.

WITH his tongue in his cheek, Thomas Henry Huxley once wrote in a preface to an account of his life, "Autobiographies are essentially works of fiction". Of course, his own was not fictional at all, and nor is his grandson's, for style and artistry help and do not hinder truth. It will be salutary for those who only know of Sir Julian as the leading British biologist to realize how very much more than the leading British biologist it is necessary to be in order to be the leading British biologist. Particularly interesting is the part in his life which poetry and beauty play, instilling in him much intenser feeling than any church service. Intellectually hostile to orthodox Christian dogma, he is all the more deeply religious without revelation, and rejects the excessively materialistic standpoint of certain so-called rationalists.

Many of his pages are contributions to literary history, such as the party at Les Diablerets, when Aldous's wife Maria typed out D. H. Lawrence's *Lady Chatterley* (he and Frieda were there), while Juliette typed out Julian's *Science of Life*. Then there were his experiences of that tiresome genius H. G. Wells, and his exposure of the abysmal and arrogant ignorance of Bernard Shaw in biological matters.

Julian sets high store by intuition when deciding on a line of research, and it is fascinating to see how his bird watching led through observation of common birds to the mating habits of redshank, grebe, heron, and many others, discovering the principles of ritualization and displacement activities, and the fact that the birds' behaviour can only be satisfactorily interpreted on the view that they experience emotion and fun. His work published in 1914 was a turning point in the science of ethology.

He had plenty of other lines of research: differential inhibition and re-differentiation in polyps, sea squirts, and metamorphosing sea urchin larvae; experimental embryology and the field concept; allometric growth; genetics; "new systematics" integrated with genetics and ecology, and introducing the concept of the cline, the grade and the clade; and evolution, his greatest work. He is the only Fellow of the Royal Society to have made a film ("The Private Life of the Gannet") which was awarded an Oscar.

One of the secrets of his outstanding career is his physical courage. It takes nerve to climb the back of Balliol Hall, the North Face of Trinity Clock (setting the hands wrong), the Ashmolean, and, symbol of anticipated success, the Examination Schools. This is further seen in his mountaineering; Aiguilles Dorées, Grivola, and Col du Géant in the Alps, Karissimbi in Africa. It also takes nerve when past youth to climb into the crow's nest of a 60-ton sealer on the way to Spitzbergen and to camp out there, to sail to St Kilda in a small yacht, and to run the London Zoo during the blitz. This introduces the infamous, scandalous behaviour of the old zoo council in trying, illegally, to dismiss him from the secretaryship, and then making it impossible for him to do anything other than resign.

Few biologists have had as many friends of all kinds, with whom he has practised cross-fertilization of ideas. Without mentioning any in Britain (except his better half who has helped and supported him beyond compare for more than half a century), they include all the great American biologists, Judge Holmes, Judge Frankfurter, Richard Goldschmidt, Richard Hertwig, Dohrn, de Vries, Benedetto Croce ("who looked more like a prosperous butcher than a savant"), Norbert Wiener (pioneer of cybernetics), Berg, Zawadovsky, Bukharin, Lunacharsky, and the blind and deaf Helen Keller (whose contact with the world was through the sensitiveness of her fingers) whom he saw feeling a sculpture by Michelangelo. There are not two cultures for Julian. Well does he say, "The era of solitary exploration in the laboratory or the field is now largely a thing of the past, but it was not so in my earlier days". Nor in mine.

The number of adventures that have come his way is remarkable: to be asked by a German doctor in Moscow for a copy of *The Science of Life* as a fee, and by a Lebanese pawnbroker in Kumasi for a copy of *Life and Letters of Thomas Henry Huxley* in return for some gold ornaments. He was present at a tirade by that unspeakable pseudoscientist Lysenko, Stalin's biological Rasputin, and he could "boast of having been one of the few people to have dined with Stalin in the Kremlin one night, and had tea with my wife in Hampstead next afternoon".

One of the duties of a scientist is to fight superstition, and there is a splendid description of the exposure of a medium who pretended to summon St Theresa to communicate messages from the departed. The medium was found to be in a cheese cloth, tried to hide a phosphorescent plate, and then shammed dead. He signed a confession of fraud, but within a few weeks was performing again before another audience of fools who were soon parted from their money. The obstinacy of the will to believe is itself a study.

Slightly different was the episode of Julian's advocacy of birth control on the BBC. Lord Reith abused him for having profaned "his" ether with such disgusting stuff. This topic was by no means disgusting to William Temple, Archbishop of York, who wrote to Julian, "I should cordially agree with you that birth control is just as much and just as little 'unnatural' as a pocket-handkerchief or false teeth". As Julian says, John XXIII would not have been scandalized by the report of the Lambeth conference; now the old Italian bachelor who knows no biology but decides off his own bat how hundreds of millions of men and women all over the world shall conduct their sex life, sets the stage again for the old battle between revelation and science. Julian carries on the fight which his grandfather called The New Reformation, and the spirit of Galileo, of Darwin, and of Thomas Henry Huxley still has to be vigorously defended against obscurantist superstition.

It must be a continuing source of satisfaction to Julian to see how the projects which he has either initiated or recommended are flourishing, like the Arts Council, the Council for Industrial Design, Political and Economic Planning, Planned Parenthood, the universities of Makere, Ibadan, Achimoto, Freetown, various British national parks and nature reserves, and the United Nations preparatory commission. But here I must stop, because the book does; in the sequel (to which we look forward eagerly) the world will be reminded of who it was who put the S in UNESCO.

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LATE LEARNERS

A History of Adult Education in Great Britain

By Thomas Kelly. Second edition. Pp. xii + 420. (Liverpool University Press: Liverpool, 1970.) 63*s*.

THIS is the second edition of a work first published in 1962. It has been enlarged by the addition of chapters on the

Second World War, the post-war pattern, and what is called "The Four-Fold Partnership"—of the central government, the local education authorities, the universities and voluntary organizations. It is a work of considerable scholarship and readers not familiar with adult education may be surprised by the wealth of literature on which the author has drawn.

The story is complicated, and many institutions and movements are described which have long since disappeared. Of those active today the universities have been longest in the field. The starting point was in 1867 when James Stuart, who subsequently became the first Professor of Mechanism and Applied Mechanics at Cambridge, began giving courses of lectures, chiefly on astronomy, to audiences in the north of England. From them developed a national system of university extension, which has continued to the present day and has shown itself to be notably adaptable to changing social and educational needs. The most recent development mentioned by Professor Kelly is the appearance of the technological universities as providers of adult education courses. Unlike the traditional universities, Bath, Strathclyde and Loughborough have established programmes of work without the assistance of special grants from the central government. That they are specially interested in applied science is significant.

While the record of the universities and the Workers' Educational Association is of continued growth, that of the local education authorities, providing for a much larger number of people, is less satisfactory. The statistics given by the author show that their adult education activities are particularly vulnerable to public economies: expansion seems inevitably to be followed by contraction. This is because their chief purpose is the education of the young, and adult education becomes the first activity to suffer when cuts have to be made.

Readers of *Nature* will be interested in the information provided about science in adult education. It started with classes in mathematics and astronomy in connexion with the study of navigation in the 16th century, produced itinerant lecturers in the 18th century, inspired the establishment of mechanics institutes in the early 19th century, and affected the foundation of university extension through James Stuart. The record was not so satisfactory in the period before the Second World War, but the number of courses in science provided by universities increased from 6 per cent of all courses in 1947–48 to 14 per cent in 1966–67. Professor Kelly points out, however, that Bristol has a much better record than any other university and indicates that this is a result of policy rather than of accident.

Science seems to be needed, however, in another respect. Many of the movements the author describes have been the result of hope and belief, rather than a careful assessment of needs, resources, and possibilities. The general expansion of education, the start of the Open University and the report of the committee now sitting under the chairmanship of Sir Lionel Russell should enable planning in the future to be more systematic than in the past.

W. E. STYLER

LANGUAGE-LACK LORE

Aphasiology and other Aspects of Language

By Macdonald Critchley. Pp. ix + 405 + 12 plates. (Arnold: London, March 1970.) 180*s*.

APHASIOLOGY is a portentous—and etymologically somewhat bizarre—word, softened on the dust cover of this book by juxtaposition with a charming picture of a pair of gannets communicating, whose previous intercourse has evidently been rewarding to judge by the chick at their feet. Within are to be found assembled all Dr Critchley's contributions on topics relating to speech and its disorders, with excursions ranging far into surrounding byways, and amusing historical touches. Comprising some quarter of a million words, this is a treasure house for anybody not wholly enslaved by a purely clinical or a coldly scientific approach to its nominally central theme.

As in all books of this kind, with varied contents written over a considerable period of time, and some inevitable repetitions, it is difficult to discern clear lines of systematic thought and committed formulation. The author does much to provide a stimulating, and corrective, context for others to think in. Topics such as the relation of animal to human communication, iterations and verbal tics, regional and demotic speech, gestural and mimic language, the details of disordered written language, the speech of psychotics and the varied significances and euphemisms for the words "Yes" and "No" should surely all form part, if peripherally, of the framework within which the neurologist, the linguist and the psychologist must try to tease out of the rich picture of clinical phenomena definite issues on which to base hypotheses suited to decisive test. Perhaps the most valuable and suggestive theme is the author's insistence on the importance of far more careful study of what he calls "minimal" aphasia. The phenomena associated with major disorders of speech are often so dramatic and compelling that theoretical views have been framed largely in terms of them, to the exclusion of what may be learnt, and perhaps more fruitfully thought about, from minor abnormalities to be found in the residual symptoms of long standing cases, in lesions exclusively of the non-dominant hemisphere and in disorders of uncertain aetiology such as "specific dyslexia" and "dysgraphia".

This is a very long book, more suited perhaps to leisure-time browsing than intensive study; sadly its price will put it out of range of many who would like it on their own shelves. Is it perhaps ironic that one who has devoted so much effort and so many words to the study and alleviation of speechlessness should now declare: "Words which should be servants now become masters. A veritable tyranny of words develops. A *pot-pourri* of clichés may eventually usurp the place of intellectualism, beliefs and judgments. Such is one of the insidious weaknesses of the structure of modern life, threatening to be an even greater menace in the future"? Can any neuropharmacologist oblige with a contralexic pill?

R. C. OLDFIELD

PATHOLOGY, PAST AND PRESENT

Trends in Clinical Pathology

Essays in Honour of Gordon Signy. Pp. xi + 352 + 4 plates. (British Medical Association: London, February 1970. Published for the Association of Clinical Pathologists.) 60*s*; $7.50.

THIS volume is a collection of essays written by distinguished British pathologists to honour Gordon Signy on his twenty-fifth anniversary as editor of the *Journal of Clinical Pathology*, the official journal of the Association of Clinical Pathologists.

Twenty-five years ago clinical pathologists were expected to be masters of most if not all hospital laboratory investigations. But new advances in medical knowledge and the increased complexity of diagnostic methods (plus the ever expanding demand for them in the National Health Service) had produced a new breed of scientific pathologist. This is readily appreciated by reference to the scope of the papers that filled Signy's journal over the years, or by reading the essays collected here in his honour.

The essays range through the major disciplines of pathology: histopathology, medical microbiology, haematology and chemical pathology to the newer clinical importance of immunology, cytology, automation and computers in the laboratory. Some contributors take a historic approach to developments in their subject over the past two or three decades, review its modern status, and speculate on future prospects. In this respect, this volume reviews a changing and expanding scene in the clinical laboratory. New developments in chemical pathology such as microtechniques related to paediatrics (Barbara Clayton) and the swing to automated computerized analysis in large central laboratories (T. P. Whitehead) are placed in perspective by N. F. Maclagan in his essay on "Chemical Pathology at the Crossroads". Haematology, present and future, is reviewed by E. K. Blackburn and J. V. Dacie, and G. H. Tovey tells a fascinating story of the developments in blood transfusion from wartime days to the presently available range of blood products. The role of microbiology in an altering disease pattern is further emphasized by J. A. Dudgeon, L. P. Garrod, Joan Taylor and R. E. O. Williams.

Other contributors concentrate chiefly on their research interest such as haemostasis (Rosemary Biggs), atherosclerosis (T. Crawford) and the relation of fibrin to hyalinosis (A. C. Lendrum). The latter two essays are accompanied by the best series of colour illustrations that I have seen in any book.

But all of the emphasis on clinical laboratory advance during twenty-five years is then brought down to earth by R. J. V. Pulvertaft, who writes on some of his experience with new and old diseases in Africa and the problems, apart from medical, facing those who investigate them.

This, by its very nature, is a once-and-for-all book, but for those who, like Signy, have lived and worked through this emergent period it presents a fascinating account of what we have and what we might expect in the future. J. L. Stafford, who organized its publication, is to be congratulated.

W. A. J. CRANE

EPILEPSY

Basic Mechanisms of the Epilepsies

Edited by H. H. Jasper, A. A. Ward and A. Pope. Pp. xxiv + 835. (Churchill: London, 1969.) 250*s*.

EPILEPSY has always been something of a mystery, but if the chief problem is still unsolved, at least this is not a subject that is standing still. Important advances in recent years have come from the electrophysiologists and electroencephalographers, in interpreting the different types of seizure pattern revealed in the clinical electroencephalograph.

The field of investigation has now been further widened, and in *Basic Mechanisms of the Epilepsies* we are presented with an encouraging range of information obtained by a variety of new techniques. The pharmacological approach has been extended by microelectrode studies of the action of anticonvulsants on individual cells. Our anatomical concepts must now include the world of ultramicrostructural detail revealed with the electron microscope, and it is significant that nearly half the chapters in this volume are dealing with neurochemical aspects of the problem.

The book started as an attempt to produce "a modern definitive statement of current knowledge regarding fundamental aspects of the epilepsies in the form of a comprehensive monograph". A meeting of experts was duly arranged at Colorado Springs, and the result is a volume of thirty chapters, to most of which has been added an additional contribution by a designated discussant. The general standard is as high as is to be expected from the very distinguished group of contributors, most of them well known in the field of brain research. The book is well illustrated, well indexed and well produced, a credit to all who have taken part. It can be recommended as the best current collection of recent information about the various aspects of brain research which have a bearing on the problem of epilepsy.

DEREK RICHTER

PATHOLOGICAL PIGMENTATION

Pigments in Pathology

Edited by Moshe Wolman. Pp. xvii + 551. (Academic Press: New York and London, September 1969.) 275*s*.

IN his preface the editor has this to say: "In various branches of science the investigator seems to pass through two phases which are distinct though intermingled. The first is a descriptive, cognitive phase in which both static and dynamic phenomena are observed and studied. These observations might be of natural phenomena or of experimental setups. The second phase is one of understanding the mechanisms involved and the implications and significance of the phenomena. A description of deposition of various pigments in different organs and tissues is of importance, even if this deposition is of no pathological or biological significance. The understanding of the various mechanisms underlying pathological pigmentation, of the implications of these processes as expressions or indications of the basic metabolic derangements, and of the deep meaning of these phenomena seem to be of greater interest and importance than the mere description. The following chapters attempt to do both to the best of the ability of the authoritative contributors."

With this clear and simple statement of editorial wisdom and intent, it becomes apparent that the contributors sailed under a sure captain. Unequivocally and without exception they have precisely fulfilled the task he set them.

After reading the opening chapter (Gedigk on "Pigmentation caused by Inorganic Materials"), the first comment I jotted down was "This is an example of a perfect chapter". It has an introduction for the benefit of the uninitiated, a middle and an end. It presents its subject clearly and concisely and pursues it without deviation in progressively greater depth, so that the reader can either follow it all the way or he can stop short and still receive a sound general coverage of it. The layout is clear, the prose is freely readable and there is a very ample bibliography. A chapter with these qualities from an author who is right on top of his subject leaves nothing more to be desired. And this opening chapter is not unique. At the end of the second (Heppleston on "Pigmentation and Disorders of the Lung"), I wrote simply "Another perfect chapter". And so it went on throughout all the sixteen chapters in the book. Some of these concern small new topics (for example, Saxén on "Tetracycline Pigmentation"), while others deal with huge and age-old subjects (for example, Liban on "Pigmented Naevi and Malignant Melanomas"). But no matter what the scope or status of the subjects, the excellence of the articles about them is the same.

To comment individually on each chapter would be a pleasure which space does not permit, and to single out a few would be unfair to the others. This book should be in every library used by pathologists and preferably in every pathology laboratory; certainly in all those where young pathologists are trained. And if a reviewer may be allowed one wish, it is that this book might be made compulsory reading for all those editors and contributors who contemplate taking part in the production of future multi-author works.

Professor Wolman and his team of authors are to be congratulated on producing an authoritative coverage of a field which has not been synthesized in this way before and on making it fascinating reading. They are also to be thanked for proving to all those reviewers, librarians and readers, who by now must inevitably have become cynical about the flood of symposium proceedings and other edited collections now being published, that such a work can be worth more than the enormous price which has to be paid for it.

I. W. WHIMSTER

DEVELOPMENTAL IMMUNOLOGY

Immunology and Development

Edited by Matteo Adinolfi. (Clinics in Developmental Medicine, No. 34.) Pp. 187. (Spastics International, in association with Heinemann (Medical): London, 1969.) 63*s*.

THIS slim volume, approaching developmental immunology from many directions, is an interesting blend of fact and fantasy. Opening with Talmage's expert account of clonal selection it presents the reader with more hypotheses per page than is usual, even in immunology. Adinolfi and Wood then contribute a thorough and elegant chapter on the ontogeny of immunoglobulins and complement in man, providing a lucid and factual account of their important research which is likely to be of considerable interest to clinician and pure scientist alike. The following chapter is more provocative. Written by Clem and Leslie, it is an entertaining review of present knowledge of the phylogeny of the immune response. Starting with speculation on immune mechanisms in plants, these authors climb the evolutionary ladder in a thorough and very readable way, pausing inevitably at the hagfish. Although pleasing to hear from them that the standards of hagfish husbandry have been improved it is saddening to be told that this fine and noble creature has been deposed from its pinnacle in immunology. It seems that it can reject skin allografts after all.

The influence of the late David Kirby on developmental immunology is clearly emphasized in the chapter by Billington on the basic immunological enigma of pregnancy. Why is the foetus not rejected as an allograft? No complete answer is available, but in reviewing the possibilities Dr Billington communicates the fascination of this problem. One can perhaps quibble with minor details of terminology, always a topic for disagreement among immunologists. The word homograft, a misnomer, is now obsolete. The term "maternal tolerance" is used several times. The phenomenon it describes is not immunological tolerance in the classical sense and it would seem safer to substitute some other expression. Billington also incorrectly assumes that the transplantability of human choriocarcinoma in a xenogeneic host implies the absence of transplantation antigens from the tumour.

A chapter by Hobbs reviews the causes of primary immune paresis in man and, although almost unreadable, it is a powerhouse of condensed factual information which could become a very useful source of reference for practising clinical immunologists and paediatricians. McConnell, in the final chapter, concentrates on another clinical aberration of immunology and development, incompatibilities of red cell, white cell and platelet antigens between foetus and mother. He provides a suitably optimistic note by emphasizing the preventability of Rh sensitization with anti-D serum.

The wide range of subjects covered and the variety of approaches to them illustrate both the fascination and clinical importance of developmental immunology. Whether one is concerned with the immunology of development or the development of immune responses this book is worth reading. Dr Adinolfi has assembled it with care. It is short, well produced and appears in a very handsome jacket.

G. A. CURRIE

INTIMATE RELATIONS

Parasitism and Symbiology

An Introductory Text. By Clark P. Read. Pp. vii + 316. (Ronald Press: New York, 1970.) n.p.

THE word symbiosis can have several meanings and Dr Read uses it in this book to describe the intimate interrelations between different species of organisms irrespective of whether the association is harmful or not. Parasitism is therefore considered as a form of symbiosis.

Parasitism is a way of life common to many plant, animal and microbial groups, but for historical reasons the phenomenon is rarely studied as a single entity. The aim of the book is to cut across artificial boundaries and to demonstrate the basic principles of the topic. In order to do this, examples are chosen from bacteria, viruses and parasitic fungi as well as protozoa and helminths. While this is entirely laudable, the breadth of the subject and interdisciplinary approach are probably too great for a single author to do full justice to all aspects of the subject. The coverage in some chapters is therefore uneven. Indeed, the preponderance of material in the book as a whole reflects the author's interest in parasitic protozoa and helminths. The chapter on the genetics of symbiosis, where microbes are adequately covered, merely underlines the scarcity of information on helminths.

The initial chapters are concerned with definitions, particularly the problems of defining the term diseases Life cycles are presented in the guise of adaptations for reaching hosts, and readers will recognize much that is already familiar in the chapter. There is a need for an entirely new approach to the dynamics of the life cycle, and the description of life cycles in terms of open feed-back loops which are closed by the host could be further developed. In this context, the recent use of systems analysis to study the epidemiology of a sheep-nematode host-parasite system is given full treatment.

The sections on adaptations for establishment in the host, nutrition, and metabolism are the best in the book, reflecting the most active areas of research in recent years.

The final chapter, symbiology and human affairs, gives an unusual insight into the impact of parasites on civilization, from the plagues of the middle ages to schistosomiasis and malaria, both so important today. The principal conclusion of this chapter is that parasitic diseases will be with us in the foreseeable future because of the plastic nature of the interaction between host and symbiote and the continual appearance of drug resistant strains or virulent mutations.

The general level of the text makes this a book for the undergraduate rather than the specialist. The layout of the book is clear with numerous subheadings and excellent illustrations. Above all, the text is very readable.

R. A. WILSON

X-RAY DIFFRACTION

An Introduction to X-ray Crystallography

By M. M. Woolfson. Pp. ix + 380. (Cambridge University Press: London, March 1970.) 85*s*; $13.50.

THE number of textbooks on X-ray diffraction is increasing rapidly, and each one now has to justify itself in some special way if it is to become generally accepted. This book falls clearly into the category of an introductory work; it ties the principles of the subject to the basic physics that the undergraduate will have learnt, and it gives enough detail for the new research student to cope with the procedures that he will be faced with. It avoids the peril of including so much theory that the newcomer to the subject will feel overwhelmed. The author is well qualified for the task; he has made significant contributions to X-ray crystallography; he is a professor of theoretical physics and hence well able to cope with some of the complexities that are glossed over in other books; and he is a good experimentalist who knows the difficulties with which the embryo research student is faced.

The book contains nine chapters—on the geometry of crystals, the scattering of X-rays, crystal diffraction, Fourier-transform theory, experimental methods, intensities of X-ray reflexions, space-group theory, determination of crystal structures and refinement. There are forty-four problems at the ends of the various chapters, carefully chosen and nicely graduated in complexity; solutions are given at the end of the book.

Of course, no author will ever accept that another's book is ideally organized, and, true to this tradition, I shall give my views on what I think are some of the deficiencies of the book and how they could be corrected in future editions.

First, the treatment of symmetry is too brief. The subject seems easy to experienced workers, but new students do find difficulties with its principles and conventions and they need a gradual introduction. A particular defect in the book is the absence of a treatment of two-dimensional space groups; symbols such as pgg are introduced later in the book without any explanation. No attempt, also, is made to show the derivation of three-dimensional space groups; they are dealt with purely descriptively. Translation itself is regarded as a symmetry element, centred lattices resulting from this operation rather than from the combination of parallel symmetry elements such as mirror and glide planes.

The section on diffraction starts off oddly, with a rather schoolboyish derivation of the diffraction pattern of a grating; the complex-exponential method would have fitted in better with the succeeding work. Also, the relation between the Fourier transform and diffraction theory is too brief; experience has shown that it needs careful exposition if it is to be properly appreciated. The same criticism applies to the introduction of the reciprocal lattice; it is treated purely as a mathematical contrivance and its basic physical meaning is not mentioned.

On the other hand, the chapters on the scattering of X-rays by atoms and the intensities of X-ray reflexions are excellent. The author shows considerable physical feeling for these subjects, and any reader who properly digests these chapters will have a better appreciation of the basic principles of the subject than many experienced crystallographers have. The theories of absorption, extinction, temperature effects and anomalous scattering are treated much more effectively than in most introductory textbooks.

The book is well produced, and the diagrams especially are beautifully conceived and drawn; the author has clearly spared no pains in expressing his ideas through them. The writing also, apart from a few grammatical infelicities, is very good and clear. The book can be recommended unreservedly to those contemplating entry into this still fascinating field of the study of crystalline matter through its diffraction of X-rays. H. LIPSON

LIQUID CRYSTALS

Liquid Crystals and their Applications

By Thomas Kallard. Pp. vi + 219. (Optosonic Press: New York, 1970.) $12.

DURING the past ten years, the subject of liquid crystals has changed from one of almost purely academic interest to one of considerable technological importance. This book now provides a source of valuable information on many of the technological applications of nematic and cholesteric liquid crystals. This is achieved by presenting detailed accounts of twenty-five patents relating to liquid crystals and their uses. These include the use of nematic liquid crystals in electro-optical devices such as valves, shutters, visual display systems, phase-matching devices for light beams, multi-frequency light deflectors, memory systems, and the like, and also of nematic liquid crystals containing dichroic dyes for colour display systems. Other patents describe the uses of cholesteric liquid crystals in devices for thermal imaging, in detecting electric fields, in detecting low concentrations of vapours, in surface thermography, for example, of skin, and in non-destructive testing of electronic circuits, laminates and so forth. Patents relating to the preparation of polarizing films and sheets, encapsulated cholesteric liquid crystals and dried emulsion coatings of cholesteric liquid crystals are also given. The contents therefore give a broad impression of the implications of liquid crystals in spheres such as medicine, television, electronics and engineering. A brief list of areas of possible future applications is also interesting.

The book does not provide basic background information on liquid crystals, but for the reader who requires this, there is presented an extensive bibliography of texts, review articles and publications relating to liquid crystals. This adds value to the text by drawing together many widely disseminated sources of information.

The reader should realize that the bibliography, while extensive, is not exhaustive, particularly with regard to literature before about 1960. The text is pleasing in presentation, but a little marred by typographical errors.

Readership should extend to chemists, physicists, medical technologists, engineers and electronic engineers. By appealing to such a broad group, the text should stimulate yet more interest in liquid crystals, and if this in turn leads to further beneficial applications, publication of the book will have been amply justified.

G. W. GRAY

HIGH ENERGY PHYSICS

Proceedings of the Fourth International Symposium on Electron and Photon Interactions at High Energies

Edited by D. W. Braben. Pp. ix + 328. (Daresbury Nuclear Physics Laboratory: Daresbury, nr Warrington, 1969.) 120s.

SCIENCE becomes ever more specialized. The first International High Energy Physics Conference (Rochester Conference) was held in 1955. At the 1960 conference a small subgroup of physicists working—or hoping to work—with electron accelerators met for one day at Cornell University to discuss their specialized topics. This became a habit; a separate symposium on electron and photon interactions was held in Cambridge, Mass., in January 1963; others at DESY, in Germany, in June 1965, at SLAC, in California, in September 1967 and now another at Daresbury, in England, in September 1969. Each occasion accelerated the coming into operation of a new electron accelerator. Many more physicists now work in this specialized field and produce exciting work. The conference was unusual in that individual papers were not read at all, but summary talks were presented, summarizing the recent work in the field including the submitted abstracts. This makes the conference proceedings easier reading for the inexpert.

A vast quantity of new information was presented on various photoproduction processes. But the exciting new information lies in three fields: inelastic electron scattering, the study of vector mesons (ω, ρ and φ) particularly in their production by electron–positron storage rings, and in quantum electrodynamics.

Inelastic electron proton scattering has been studied for some time, but Dr R. E. Taylor, of SLAC, presented his experiments on inelastic scattering with energy transfers of several BeV. Unlike the elastic electron proton scattering, and the scattering leading to definite excited nucleon states, this "deep" inelastic scattering varies only slowly with momentum transfer. This therefore describes a structure at distances smaller than the proton size. Various theoretical attempts to describe this have been made; it seems that the electrons are scattered by a small part of the nucleus; the constituent is commutally called a "parton".

The vector meson production by electron–positron colliding beams has been extended in the last year. There now exists good measurements on the ρ meson, detected by its usual $\pi^+\pi^-$ decay; the ω, detected both by its $\pi^+\pi^-\pi^0$ decay and a rare $\pi^+\pi^-$ decay; and the φ detected now by K^+K^-, $K_1^0\,K_2^0$ and $\pi^+\pi^-\pi^0$ decays. All of these give a consistent picture. The coupling of each vector meson to the photon field is measured and seems to be, within errors, equal to that assumed in the model of Vector Meson Dominance (VMD), first proposed by Sakurai, in which the ω, ρ and φ mesons are assumed to dominate the electromagnetic interactions. Dr Alcock presented one of the first experiments from the Daresbury Laboratory showing the interference between the leptonic decays of the ω and ρ mesons. Much work has been done in the past year to establish, both theoretically and experimentally, the limits of validity of the VMD model. Deviations are observed but VMD remains a very useful concept.

Experiment and theory now agree that quantum electrodynamics describes purely electromagnetic processes down to distances of the order of 10^{-15} cm. At the conference, Dr Brodsky described the recent advances; experiments of bremsstrahlung and pair production by electron and muons at high momentum transfers agree well with calculation; the difference of the g factor of the free muon from the Dirac value of 2, measured at CERN by Dr Farley and collaborators, now agrees with theory. A recent calculation of a correction term by Dr Kinoshita and collaborators—a term involving scattering of light by light—brings a new and welcome agreement. Since the conference, and therefore not in the proceedings, a recalculation of the 4th order corrections to the Lamb shift has been made, which removes another discrepancy. Everybody in the field will need a copy of these proceedings near them (till the next conference) and others would do well to read it.

Richard Wilson

NEW MATHS

A Comprehensive Textbook of Classical Mathematics

A Contemporary Interpretation. By H. B. Griffiths and P. J. Hilton. Pp. xxix + 637. (Van Nostrand Reinhold: London and New York, February 1970.) 120*s*.

This is a splendid introduction to the "new maths". "Comprehensive" is a bit grand—the material is more or less at first year undergraduate level, and the book grew from a course of lectures to school teachers, who wanted to be up-dated with the new language of sets and functions. "It has not been our primary intention to offer readers their *first* contact with the concepts and ideas we treat," so that the course is heavily didactic. The authors are not against discovery methods; the creative aspect is important, but here they are jolly well telling us how one acquires strength through unification. They have ducked the important question as to how far the new notations and treatments might be used for the actual introduction of topics, at school level, but perhaps they can reasonably argue that this is not their problem.

The book is in eight parts. The first two set up the language of set theory, and the last introduces categories and ends with a chapter on mathematical logic. In between, first, there is arithmetic, generalizing from the integers to rings with a norm. Next, there is three-dimensional geometry, introduced with vectors and leading on to linear algebra. The final chapter of this part is devoted to projective geometry. Durell would not have recognized the language, but the authors justify their "commercial" at the beginning for the excellence of projective geometry in showing what genuine mathematics is. (Incidentally, the definition of cross-ratio "where $|PQ|$ here is $-|QP|$" reads a bit strangely—this seems to arise from an ambiguity on p. 188.) Part five is devoted to algebra (groups, vector spaces, Boolean algebra, polynomials); and part six to number systems and topology (successive build-up to complex numbers and on to metric spaces).

The remaining part seven is concerned with calculus. The authors are at the same time humane teachers and puritanical mathematicians. The reader is edged forward with the spiral approach, but extrinsic motivation is minimal (the logarithm, for example, is starkly defined as an integral). But then this is a course for the already converted. Is there any danger that the fascination of compact language will lead, not to unification and clarity, but to confusion ? Certainly no schoolboy at the stage of discovering that every differentiable function is continuous is going to thank his teacher for the theorem:

> D(I) is a sub-algebra (over R) of C(I), containing the constants and the identity function I.

Will even undergraduates appreciate this particular formulation ?

But, by and large, the new look has been amply justified and agreeably presented. The above Michelin-type summary can give no real impression of the scholarship and enjoyment clearly shared and imparted by the authors.

Geoffrey Matthews

ELEMENTARY EQUATIONS

Elementary Differential Equations

By R. L. E. Schwarzenberger. (Chapman and Hall Mathematics Series.) Pp. xii + 98. (Chapman and Hall: London, September 1969.) 20*s*.

This introductory textbook is based on a course of twenty lectures given at the University of Warwick to first year students. The motivation is clear and admirable: to base the course on geometric intuition and to be concrete rather than abstract. The former is in the spirit of a great deal of contemporary research in the field of differential equations and the concrete approach achieves two objects; first, it means that the course is more directly related to school mathematics, as experienced by most pupils, and second, it leads, in a natural manner, to various abstract notions such as continuous function, vector space and linear operator.

The exposition commences with an account of differentiable functions and with some examples of differential equations. A large number of diagrams illustrate the text, this being in the spirit of the geometric approach. Then the solution of first order equations is discussed thoroughly. Possibly the most interesting and important chapter is the one relating to autonomous systems. The frequent occurrence of such systems in physical contexts is pointed out and the geometric representation of the solutions is emphasized.

The book closes with a guide to further reading, particularly in the mathematical theory, and exercises are set at the end of each chapter. The student should find the book eminently readable, and it is reasonably priced. It is refreshing, in view of the large number of texts in recent years on the subject of differential equations, to find one that is different in emphasis from most of the others.

L. S. Goddard

Correspondence

Smiles at the Unobtrusive

SIR,—A second round of supercilious comment on my quantitative estimates of scientific manpower (*Nature*, April 4, 1970, p. 10) leaves me wondering about the status of my unknown assailant. It reminds me of the regrets expressed long ago in these columns that perhaps the editor of *Nature* was being confused with its author. At all events I protest the imperious editorial attitude and remind you that the protecting shelter of anonymity carries an implied responsibility in speaking for the British scientific community. One would have no objection to a signed statement by a mere person who might find that my (or any other) social science investigation of scientists was distasteful or even loathsome. Indeed, the resistance of scientists to such intrusive examinations of themselves is well known, and though it is not pleasant, it has been a most illuminating and revealing topic of serious discussion and analysis by sociologists of science. With an unsigned editorial it is different. It surely ill behoves scientists in Britain to countenance an official attitude of this sort towards serious social science investigations into such things as the geographic distribution of scientific manpower, and any regularities and laws there might be connecting such parameters with other demographic variables. Such investigations have long exercised my respected colleagues in several countries and are hallowed by Unesco and OECD. British science policy is neither so competent and well managed nor so replete with reliable statistics that the establishment can afford just to smile at things which may be learned from these unobtrusive indicators of the deployment of our resources.

I ignored the first round of editorial humour (*Nature*, **217**, 793; 1968), supposing that its purpose was merely to amuse the readers or to draw me into a personal controversy in which opinions of scientists about science might be entertaining. My attitude towards my work is, however, not much different from that of a physicist or a chemist towards theirs. I do not publish with the principal objective of debate for an amusable public, but in the hope of adding something to knowledge before the audience of peers. If *Nature* contains on its editorial staff or among contributors those who are seriously critical and competent in the matters of estimates of scientific manpower and literature, let them speak and be very welcome indeed. The concept of a "publishing scientist" is open to a great deal of uncertainty but it gives a consistent body of reasonable laws and hopefully a rather useful analysis of a difficult problem that I would be happy to see discussed. If not, I think that the editor of *Nature* would be well advised to check the boisterousness of that part of himself that would poke fun at the antics of social scientists. The tradition of William Petty to actuarial arithmetic is not to be mocked by those who are that contemptuous of their colleagues as to think it stupid to count anything so individual and so beyond the reach of science as a human being or a "publishing scientist".

Yours faithfully,

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New Haven, Conn. 06520.

Mediocrity of Ks

SIR,—N. W. Pirie (*Nature*, May 23, 1970) is wrong in supposing that the learned societies discriminate against alphabetical tail-enders. An inspection of the London Telephone Directory—whose subscribers we presume to be an alphabetical cross-section of London's population—has a mid-point approximately at "Kingsley" (towards the end of the Ks). This is presumably because of the infrequency of names beginning with Q, U, V, X, Y and Z.

The mid-points of the lists of members of the learned societies mentioned by Pirie therefore occur where they would be expected to (allowing for some statistical variations expected in small samples) were there no alphabetical discrimination.

Yours faithfully,

R. BROWN
R. WEST

National Institute for Medical Research,
Mill Hill,
London NW7.

Divisions among Kings

SIR,—Mr Pirie (*Nature*, May 23, 1970) suggests that there is a forward shift in the alphabet in name lists of persons recruited on the basis of apparent merit. This suggestion was based on a comparison of the list of members of the Royal Society and the National Academy of Sciences (mid-point in the Ks) with the membership lists of the Biochemical and Nutrition Societies (mid-point in the Ls). It is true that one would not expect to find selection in the latter case, and this prompted me to look in other, presumably unbiased, lists that happened to be handy, with the following results: Institute of Physics and Physical Society 1966 membership list, mid-point KING; Institute of Metals 1964/65 list, KHAN; resident members of the University of Oxford 1968/69, men KING, women KENT; the Oxford Area telephone directory 1969 (which includes businesses and institutions), KINGFEAST. The name KING is again found by opening in the middle the 3360 page *Who's Who 1958*.

The 1969 list of fellows of the Royal Society, excluding royalty and foreign members, shows that the mid-point is at KENT, so there is, after all, surely nothing to suggest that there has been any alphabetical bias in the selection of the fellowship. What is peculiar is the apparent backward shift into the Ls of the membership of the Biochemical and Nutrition Societies as reported by Mr Pirie.

Yours faithfully,

G. L'E. TURNER

46 Hamilton Road,
Oxford.

Dr Bruce and Astrophysics

SIR,—In two reports published by the Electrical Research Association in 1955 and 1958 (refs. 1 and 2) Dr C. E. R. Bruce showed that the electrification of dust in cosmic atmospheres, and the breakdown of the resulting electric fields in electrical discharges, would account for the data of astrophysics in general and the radiation from and characteristics of radio galaxies in particular. It is noteworthy that the association of the surprisingly intense radiation from quasars with dust was confirmed soon after their discovery[3], thus confirming Dr Bruce's prediction made eight years earlier. This and the theory's many other successes, many of them also demonstrably predictions, are outlined in another recent ERA Report[4], so that it is rather surprising that in their recent note Okuda and Wickramasinghe[5], while confirming Dr Bruce's suggestion as to the importance of dust in the universe, profess ignorance as to the nature of the energy source associated with it.

The novelty of many of these theoretical predictions makes their subsequent confirmation by observations all the more cogent. For example, when the highest solar temperature then discussed was only around a million degrees, Dr Bruce deduced that the temperature in solar flare discharges must reach hundreds of millions of degrees. The existence of these temperatures was confirmed by the discovery of X-rays from solar flares the following year. Dr Bruce's electrical discharge theory of solar flares would appear to have been confirmed beyond a doubt by A. B. Severny's[7] discovery, probably one of the most important astronomical observations ever made, that the transverse magnetic fields surrounding two solar flares in 1966 are such as would be caused by electric currents in the flares.

Yours faithfully,

B. C. LINDLEY

Electrical Research Association,
Cleve Road,
Leatherhead, Surrey.

[1] Bruce, C. E. R., *ERA Report* Ref. Z/T101 (*Phil. Mag.*, **46**, 1123; 1955).

[2] Bruce, C. E. R., *ERA Report*, Ref. Z/T117 (1958).

[3] Burbidge, E. M., and Burbidge, G. R., in *Quasi Stellar Sources and Gravitational Collapse* (edit.by Robinson, I., Schild, A., and Shucking, E. L., 300 and 306 (Chicago Univ. Press, 1965).

[4] Bruce, C. E. R., *ERA Report 5275* (1968).

[5] Okuda, H., and Wickramasinghe, N. C., *Nature*. **226**, 134 (1970).

[6] Bruce, C. E. R., *Nature*, **184**, 2004 (1959).

[7] Bruce, C. E. R., *Nature*, **187**, 865 (1960).

[8] Severny, A. B. (personal communication).

Announcements

University News

Dr Robert B. Leighton has been appointed chairman of the Division of Physics, Mathematics and Astronomy, **California Institute of Technology,** in succession to **Dr Carl D. Anderson.**

Professor W. J. H. Butterfield has been appointed vice-chancellor of the **University of Nottingham,** in succession to **Professor F. S. Dainton.**

Dr M. A. Stephens, McGill University, has been appointed to the new chair of mathematical statistics, **Dr R. A. Lawrie** has been promoted to the new chair of food science and **Dr P. S. Pell** has been promoted to the second chair of civil engineering, all in the **University of Nottingham.**

Dr Eric M. Wilson has been appointed to a personal chair in hydraulic engineering in the Department of Civil Engineering, **University of Salford.**

Appointments

Sir Ashley Miles has joined the board of trustees of the **Beit Memorial Fellowships for Medical Research,** in succession to **Sir Alan N. Drury.**

The Salk Institute has appointed **Dr Frederick de Hoffmann** to the new post of chancellor. Dr de Hoffmann was the founder 15 years ago of the research laboratory known as General Atomics, since absorbed by the Gulf Corporation and now known as Gulf General Atomic.

The **British Nutrition Foundation** has announced the creation of two new offices, to replace that of director-general, held by Dr Alastair Frazer until his death last June. **Professor F. G. Young,** University of Cambridge, has been appointed president of the Foundation (a part-time position), and **Miss Dorothy Hollingsworth,** who is at present head of the Food Science Advice Branch of the Ministry of Agriculture, Fisheries and Food, will become full-time director of the Foundation in August.

Miscellaneous

The Duke of Northumberland has been elected a Fellow of the Royal Society, under that statute which provides for the election of persons who either have rendered conspicuous service to the cause of science or are such that their election would be of signal benefit to the Society.

Awards from the **Ethel Behrens Fund** are available to Fellows of the **Chemical Society** who are studying for their first degree at a British university or technical college, to assist with maintenance and travelling expenses incurred in attending the annual meeting of the Society. This meeting will be held in Brighton from March 31 to April 3, 1971. Further information can be obtained from the Scientific Affairs Officer of the Society, Burlington House, London W1V OBN.

Twenty grants from the **Chemical Society's Research Fund,** each of £50, will be awarded to Fellows of the Society for the year 1971. Applications will be considered on a merit basis, but preference will be given to those working in less well endowed establishments. Further details can be obtained from the Administrative Officer, The Chemical Society, Burlington House, London W1V OBN.

ERRATUM. The contents entry for the article by N. R. Blacklow and A. Z. Kapikian "Serological Studies with EB Virus in Infectious Lymphocytosis" (*Nature*, **226**, 647; 1970) should have been **Infectious lymphocytosis,** not **Infectious mononucleosis.** In the article itself, five lines from the end, read "these children" not "three children".

International Meetings

June 29–July 3, **The Harnessing of Science and its Application to Assist in Day to Day Management,** London (Derek Osborne, Operational Research Society Ltd, 64 Cannon Street, London EC4).

July 1, 8, 15, 22, 29, August 5, 12, 19, 24, 26, **Will Rogers' Teaching and Training Institute Summer Seminars,** Saranac Lake, NY (Montague Memorial Library, Will Rogers Hospital, Saranac Lake, NY, USA).

July 15–17, **Testing Fibrous Composites for Mechanical Properties,** Teddington (Dr S. L. S. Thomas, National Physical Laboratory, Teddington, Middlesex).

July 20–24, **The Mechanisms of Reactions in Solution,** Canterbury (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1V OBN).

July 22–24, **Water Pollution Control in Cold Climates,** College (Dr R. Sage Murphy, Institute of Water Resources, University of Alaska, College, Alaska 99701, USA).

July 26–August 1, **Water Pollution Research,** San Francisco (IAWRR Registration Headquarters, Room No. 635, Davis Hall, University of California, Berkeley, California 94720, USA).

August 5–7, **Applications of X-ray Analysis,** Denver (Metallurgy and Materials Science Department, Denver Research Institute, University of Denver, Denver, Colorado 80210, USA).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Furnished 3 bedroom house in or near Cambridge for one year from August 1970. Prefer something with character rather than a modern development; will consider up to 10 miles from city centre. **Vacant:** 3 bedroom house near University of California at Irvine, for rent or **exchange** during same period. Please contact Dr C. J. Brokaw, Division of Biology, California Institute of Technology, Pasadena, California 91109, USA.

Wanted: From August 20 for 12 months, furnished flat or house with 2 bedrooms in London with easy access to the University of London, for American professor and family (3 children). Please contact Professor Jack K. Ward, Director of the Computer Center, Westminster College, Fulton, Missouri 65251, USA.

Wanted: From July 15 or August 1, 1970, until August 1971, 4 bedroom accommodation for Canadian professor, wife and 4 school age children convenient to King's College, London. Please contact Dr D. D. Betts, Physics Department, University of Alberta, Edmonton, Alberta, Canada.

Wanted: Furnished house for professor and family with three boys, in or near Cambridge, for one year from mid-August. Please contact Dr W. K. Honig, Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

Vacant: From September 1970 for 1 year (longer by arrangement), beautifully furnished, 1 bedroom, fourth floor flat (no lift) in modern block in London, W11. Please contact Dr Nigel Godson, Department of Radiology, Yale Medical Center, New Haven, Connecticut, USA.

Vacant: Furnished one-bedroom flat with kitchen and bathroom. 2 minutes from Highgate Hill. Available immediately. Please contact Frank House, 33 Ardoch Road, Catford, London SE.

Vacant: From mid-August 1970 to end June 1971, furnished country house, partial central heating, three bedrooms, 7 miles from University of Edinburgh. Please contact Dr M. Lunan, ESR Division, FAO, Rome, Italy.

British Diary

Tuesday, June 9

Effects of Fire on Vegetation and Biomass in the Akagera Park, Rwanda; Dr C. A. Spinage; **The Natural Regulation of Ungulate Populations in East Africa by their Food Supply,** Mr A. R. E. Sinclair; **Aspects of Small Mammal Ecology on Mount Nimba, Liberia,** Dr M. J. Coe; **Territory in Coke's Hartebeest in Nairobi National Park, Kenya,** Mr L. M. Gosling (5 p.m.) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Electrical Interference in Instrumentation (three-day conference) Institution of Electrical Engineers; and the Institution of Electronic and Radio Engineers, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Wednesday, June 10

Biochemistry of Photosynthesis (9.30 a.m.) British Photobiology Society, at the University of London King's College, Department of Botany, 68 Half Moon Lane, London SE24.

Pathogenesis of Infectious Processes (2 p.m.) Dr A. Glynn, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

The Reconstruction of Parts of the Central Nervous System that have become Disconnected by Disease or Injury (5.30 p.m.) Professor G. S. Brindley, University of London, at the Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5.

The Upper Atmosphere in Motion (5.30 p.m.) Dr G. V. Groves, University College London, in the Physics Theatre, Gower Street, London WC1. (Inaugural Lecture.)

Thursday, June 11

Removable Brain Tumours (5.15 p.m.) Mr Leslie Oliver, University of London, in the Page Street Lecture Theatre, Westminster Medical School, Horseferry Road, London SW1.

The Structural Basis of Muscular Contraction (4.30 p.m.) Dr H. E. Huxley, FRS, Royal Society, at 6 Carlton House Terrace, London SW1. (Croonian Lecture.)

Monday, June 15

Molecular Carriers of Ions (5.30 p.m.) Professor George Eisenmann (California), University of London, in the Botany Theatre, University College London, Gower Street, London WC1. (Further lecture on June 16.)

Role of Lipids in Membranes (2 p.m.) Professor J. Lucy, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Northern Ireland: Ministry of Agriculture. Leaflet No. 23: Hill Land Management. Pp. 16. (Belfast: Ministry of Agriculture, 1969.) [64

Bulletin of the British Museum (Natural History). Historical Series. Vol. 3, No. 7: The Reeves Collection of Chinese Fish Drawings. By P. J. P. Whitehead. Pp. 191–322 + 29 plates. (London: British Museum (Natural History), 1969.) 115*s*. [64

University of Oxford. Sixth Annual Report of the Delegates of the Science Area for the year ending 31 July, 1969. (Supplement No. 4 to the *University Gazette*, Vol. C, March 1970.) Pp. 73. (Oxford: The University, 1970.) 5*s*. [64

The Scottish Field Studies Association, Ltd. Annual Report 1969. Pp. 32. (Glasgow: Scottish Field Studies Association, 1970.) 5*s*. [74

Albright and Wilson, Ltd. Annual Report 1969. Pp. 43. (London: Albright and Wilson, Ltd., 1970.) [74

Fossils, Minerals, and Rocks. Ninth edition. (Instructions for Collectors, No. 11.) Pp. 18. (London: British Museum (Natural History), 1970.) 2*s*; 10*p*. [84

Research and Development in Mining. By Professor R. N. Pryor (Inaugural Leture, 20 May 1969.) Pp. 71–89. (London: Imperial College of Science and Technology, 1970.) 4*s*. [84

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Volume XXVI, No. 5: Studies on the Food Value of Nineteen Genera of Algae to Juvenile Bivalves of the Genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. By P. R. Walne. Pp. v + 62. (London: HMSO, 1970.) 23*s* net. [84

Proceedings of the Royal Irish Academy. Vol. 69, Section A, No. 3: A Unified Treatment of the Kerr and Vaidya Solutions in General Relativity. By M. Misra. Pp. 39–54. 4*s* 6*d*. Vol. 69, Section A, No. 4: Inner Products in Banach Spaces and Supports of Radon Measures. By T. Murphy and T. T. West. Pp. 55–62. 2*s*. Vol. 69, Section A, No. 5: Graph Theory of Weight Multiplicities. By J. McConnell. Pp. 63–76. 4*s* 6*d*. Vol. 69, Section A, No. 6: Probe Analysis of Moving Striations in Iodine. By J. R. M. Coulter, K. G. Emeleus, E. W. Gray and G. A. Woolsey. Pp. 77–83. 2*s*. Vol. 69, Section B, No. 3: The Biology of the Tench *Tinca tinca* (L.) in Irish Waters. By M. Kennedy and P. Fitzmaurice. Pp. 31–82 + plates 3 and 4. 11*s* 6*d*. Vol. 69, Section B, No. 4: The Stratigraphy and Structure of the Ordovician Rocks Between Courtown and Kilmichale Point, Co. Wexford. By P. J. Brenchley and J. E. Treagus. Pp. 83–102 + plates 5–8. 7*s*. Vol. 69, Section B, No. 5: The Food, Reproduction, Form, Growth and Development of the Fox *Vulpes vulpes* (L.) in North-East Ireland. By J. S. Fairley. Pp. 103–138. 6*s* 6*d*. Vol. 69, Section B, No. 6: The Distribution of the Irish Psocoptera. By E. D. Fahy. Pp. 139–163. 4*s* 6*d*. (Dublin: Royal Irish Academy, 1970.) [94

Research Using Transplanted Tumours of Laboratory Animals; a Cross References Bibliography—VI. By D. C. Roberts. Pp. 157. (London: Registry and Information Service for Experimental Tumours, Imperial Cancer Research Fund, 1970.) [94

London School of Hygiene and Tropical Medicine. Report on the Work of the School, 1968/1969. Pp. 168. (London: London School of Hygiene and Tropical Medicine (University of London), 1970.) [104

The Gap Widens. By Lord Blackett, OM, CH, FRS. (The Rede Lecture, 1969.) Pp. 39. (London: Cambridge University Press, 1970.) 6*s* (30*p*); $0.95. [104

The Society for Psychical Research: an Outline of Its History. By W. H. Salter. Edited, with a new section and a bibliography by Renée Haynes. Pp. 68. (London: The Society for Psychical Research, 1970.) 5*s*. [104

Bulletin of the British Museum (Natural History). Geology. Vol. 19, No. 1: Highest Ordovician (Hartfell Shales) Graptolite Faunas from the Moffat Area, South Scotland. By P. Toghill. Pp. 1–26 + 16 plates. (London: British Museum (Natural History), 1970.) 65*s*. [104

University of Cambridge. Report of the Observatories Syndicate for the year ending September 30 1969. Pp. 5. (Cambridge: The University, 1970.) [104

Ministry of Agriculture, Fisheries and Food. Fishery Investigations, Series II, Volume XXVI, No. 4: The Wash Sprat Fishery. By P. O. Johnson. Pp. vii + 88 + 6 plates. (London: HMSO, 1970.) 37*s* net. [134

Ministry of Overseas Development. Directorate of Overseas Surveys—Annual Report for the year ended 31st March, 1969. Pp. 72 + 9 maps. (London: HMSO, 1970.) 16*s* net. [134

Unilever. Report and Accounts for 1969. Pp. 48. (Port Sunlight, Wirral: Unilever, Ltd., 1970.) [144

Cotton Research Corporation. Progress Reports from Experiment Stations, Season 1968/1969. Kenya. Pp. 17. 2*s* 6*d*. Northern States, Nigeria. Pp. 37. 2*s* 6*d*. Uganda. Pp. 75. 2*s* 6*d*. (London: Cotton Research Corporation, 1969.) [144

Victorian Technology and Its Preservation in Modern Britain. By Norman A. F. Smith. (A Report submitted to the Leverhulme Trust.) Pp. 74. (Leicester: Leicester University Press, 1970.) 20*s*. [154

Comparative and General Pharmacology, Vol. 1, No. 1, March 1970. Pp. 1–128. Edited by Professor G. A. Kerkut. Published quarterly. Annual subscription £15; $40. Single copies £4; $11. (Bristol: Scientechnica (Publishers), Ltd., 1970.) [154

Air Pollution and Health. (Summary and Report on Air Pollution and Its Effect on Health by the Committee of the Royal College of Physicians of London on Smoking and Atmospheric Pollution.) Pp. 80. (London: Pitman Medical and Scientific Publishing Co., Ltd., 1970.) 30*s* net. [164

Department of Education and Science. Output Budgeting for the Department of Education and Science—Report of a Feasibility Study. Pp. vi + 170. (London: HMSO, 1970.) 30*s* net. [164

Annual Report of the Paterson Laboratories for 1969. Pp. 137. (Manchester: Christie Hospital and Holt Radium Institute, 1970.) [174

Other Countries

New Zealand: National Roads Boards. RRU Bulletin No. 8: A Review of the California Method for Pavement Design. By G. J. Tuohey. Pp. 13. (Wellington: Road Research Unit, National Roads Boards, 1969.) [243

Republic of South Africa: Department of Industries. Division of Sea Fisheries Investigational Report No. 76: Surface Currents Off the Cape Coast. By C. P. Duncan and J. H. Nell. Pp. 19. (Sea Point, Cape Town: Director of Sea Fisheries, 1969.) [253

North-Western State of Nigeria. North-Western State, Forestry Annual Report, 1967–68. Pp. 22. (Kaduna: Government Printer, 1969.) [313

Zoology Publications from Victoria University of Wellington. No. 48: The Body Wall and Musculature of the Marine Triclad *Palomniella stephensoni* (Palombi, 1938). Part 1: General Tissue Structure as seen with the Light Microscope. By J. S. Wineera. Pp. 13. Nos. 49–51: Some New Zealand Parasitic Copepoda of the Family Eudactylinidae. By G. C. Hewitt. A New Species of *Paeonodes* (Therodamasidae, Cyclopoidea, Copepoda) Parasitic on New Zealand Freshwater Fish, with a Re-examination of *Paeonodes exigiis* Wilson. By G. C. Hewitt. New Collembola from India. By J. T. Salmon. Pp. 49. (Wellington: Victoria University of Wellington, 1969.) [313

New Zealand Meteorological Service. Rainfall Observations for 1969—Station in New Zealand and Outlying Islands, including the Cook Group. Pp. 52. (Wellington, NZ: Government Printer, 1969.) [313

The Pasteur Institute of Southern India. Annual Report of the Director 1968 and Scientific Report 1969. Pp. 150. (Coonoor, S. India: Pasteur Institute, 1970.) [313

Northern Nigeria. Annual Report of the Forest Administration of Northern Nigeria, 1965/1966. Pp. 62. Annual Report on the Forest Administration of Northern Nigeria, 1966/1967. Pp. 65. (Kanuda: Government Printer, 1969 and 1970.) 3*s* 6*d* net each. [313

Académie Royale des Sciences d'Outre-Mer, Bruxelles. Classe des Sciences Techniques, NS, XVI–7: La température du Sol en Région Équatoriales Africaine. Par G. L. Dupriez. Pp. 59. (Bruxelles: Académie Royale de Sciences d'Outre-Mer, 1969.) [313

World Health Organization. Technical Report Series. No. 440: Programmes of Analysis of Mortality Trends and Levels—Report of a Joint United Nations/WHO Meeting. Pp. 36. 3 Sw. francs; 6*s*; $1. No. 441: The Pathological Diagnosis of Acute Ischaemic Heart Disease—Report of a WHO Scientific Group. Pp. 27. 2 Sw. francs; 4*s*; $0.60. (Geneva: World Health Organization; London: HMSO, 1970.) [14

New Zealand: National Roads Boards. Road Research Unit Bulletin No. 9: Transportation of Heavy-Haulage Commodities in Bay of Plenty Region. By C. Vautier. Pp. 17. (Wellington: National Roads Board, 1970.) [14

United States National Museum Bulletin No. 297: Catalogue of the Neotropical Squamata. Part 1: Snakes. Pp. viii + 347. Part 2: Lizards and Amphisbaenians. Pp. viii + 293. By James A. Peters and Roberto Donoso-Barros. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $6.75 per set of 2 volumes, sold in sets only. [14

New Zealand Meteorological Service. Miscellaneous Publications. No. 129: Upper Wind Statistics for New Zealand Stations. By J. F. De Lisle. Pp. 62. No. 130: The Climate of Molesworth. By J. D. Coulter. Pp. 22. No. 131: Climatological Observations at Makahu Saddle, Kaweka Range, 1960–66. By A. Cunningham and P. A. Gannaway. Pp. 16. (Wellington: New Zealand Meteorological Service, 1969.) [24

Durban Museum Novitates. Vol. 8, Part 17 (27 February, 1970): The Races of the European Swallow Wintering in Southern and Eastern Africa. By O. A. Clancey. (Miscellaneous Taxonomic Notes on African Birds, XXVIII.) Pp. 325–352. R 1.50. Vol. 8, Part 18 (27 February, 1970): Taxonomic Notes on the Thick-Billed Lark *Calandrella magnirostris* (Stephens). By C. D. Quickelberge. Pp. 353–362. R 0·75. (Durban: Durban Museum, 1970.) [24

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Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 139, No. 1: The Argyrolagidae, Extinct South American Marsupial. By George Gaylord Simpson. Pp. 86. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1970.) [34

US Department of Commerce: National Bureau of Standards. NBS Spec. Publ. 260–19: Standard Reference Materials—Analysis of Interlaboratory Measurements on the Vapor Pressure of Gold (Certification of Standard Reference Material 745). By Robert C. Paule and John Mandel. Pp. 18. (Washington, DC: Government Printing Office, 1970.) $0.30. [34

Field Museum of Natural History. Fieldiana: Geology Memoirs. Vol. 3, Nos. 7 and 8: The Vertebrate Fauna of the Selma Formation of Alabama. Part VII: The Mosasaurs. By Dale A. Russell. Part VIII: The Fishes. By Shelton P. Applegate. Pp. 365–433. (Chicago: Field Museum of Natural History, 1970.) [34

The Coconut Industry Board, Jamaica. Ninth Report of the Research Department, July 1968/June 1969. Pp. 86. (Jamaica: The Coconut Industry Board, 1970.) [64

Fisheries Research Board of Canada. Technical Report No. 157: Cod and Haddock Catches from Georges Bank, the Bay of Fundy, Scotian Shelf, and the Gulf of St Lawrence by Maritime Otter Trawlers in 1967. By A. C. Kohler. Pp. 26. (St Andrews, N.B.: Fisheries Research Board of Canada, Research Station, 1969.) [64

European Organization for Nuclear Research. The Isolde Isotope Separator On-Line Facility at CERN. (The Isolde Collaboration.) Edited by A. Kjelberg and G. Rudstam. Pp. x + 140. (Geneva: CERN, 1970.) [64

Commonwealth of Australia: Department of Supply. Defence Standards Laboratories Annual Report, 1968/1969. Pp. 70. (Maribyrnong, Victoria: Chief Superintendent, Defence Standards Laboratories, 1969.) [64

US Department of the Interior: Geological Survey. Bulletin No. 1286: Gold Veins Near Great Falls, Maryland. By John C. Reed, Jun., and John C. Reed. Pp. iii + 22 + 2 plates. (Washington, DC: Government Printing Office, 1969.) [64

Smithsonian Contributions to Botany, No. 3: Marine Algae of Dominica. By Wm. Randolph Taylor and Charles F. Rhyne. Pp. 16. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $0.30. [64

Die Kulturpflanze: Berichte und Mitteilungen aus dem Institut für Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften zu Berlin in Gatersleben krs. Aschersleben. Beiheft 6: Festveranstaltung au Anlass des 20. Jahrestages der Ubernahme des Instituts für Kulturpflanzenforschung in die Deutsche Akademie der Wissenschaften zu Berlin und des 25. Jahrestages seiner Gründung, vom. 25. bis 27. Juni 1968, in Gatersleben. Herausgegeben von H. Stubbe. Pp. 173. (Berlin: Akademie-Verlag, 1970.) 36 DM. [74

Smithsonian Contributions to Zoology, No. 27: The Tenrecs—a Study in Mammalian Behaviour and Evolution. By J. F. Eisenberg and Edwin Gould. Pp. vi + 138. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) $3.25. [74

US Department of the Interior: Geological Survey. Bulletin 1274–Q: The Akolai Group in the McCarthy B–4, C–4 and C–5 Quadrangles, Wrangell Mountains, Alaska. By James G. Smith and E. M. MacKevett, jun. Pp. iii + 26. (Washington, DC: Government Printing Office, 1970.) $0.60. [84

Smithsonian Contributions to Zoology. No. 32: Ostracoda (Myodocopina) from the Pery-Chile Trench and the Antarctic Ocean. By Louis S. Kornicker. Pp. 42. $0.50. No. 37: The Odonata of Dominica, British West Indies. By Thomas W. Donnelly. Pp. 20. $0.35. No. 39: Myodocopid Ostracoda (Cypridinacea) from the Philippine Islands. Pp. 32. $0.45. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) [84

Le Conseil des Recherches Agricoles de la Province de Québec. Recherches Agronomiques—Sommaire des Résultats, 1967/1968. (No. 13, 1968.) Pp. 108. (Québec: Ministère de l'Agriculture et de la Colonisation, 1970.) [84

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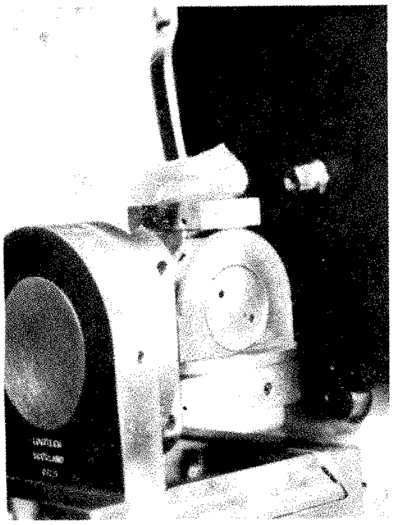

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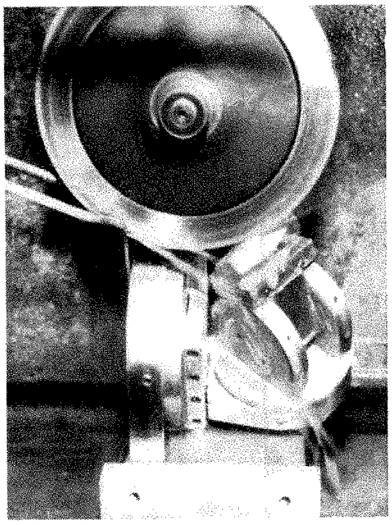
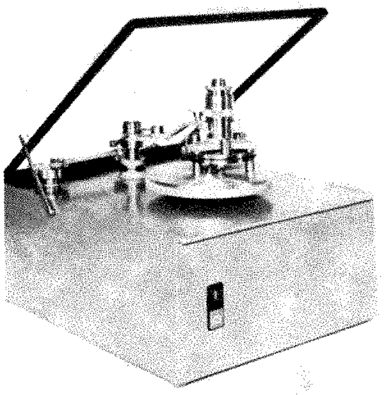


Cutting and grinding along chosen planes after angular adjustment.



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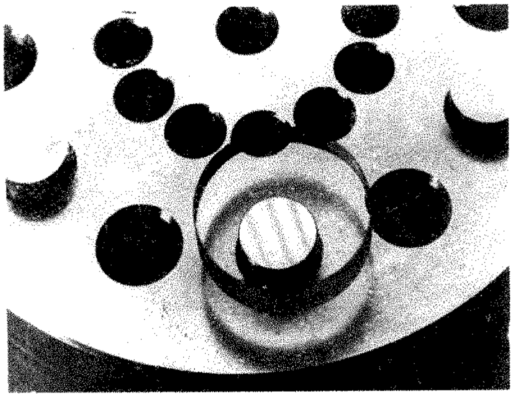

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CSIRO

REPRODUCTIVE PHYSIOLOGIST

AUSTRALIA

DIVISION OF ANIMAL PHYSIOLOGY

GENERAL The Division of Animal Physiology of the Commonwealth Scientific and Industrial Research Organization wishes to appoint a Reproductive Physiologist to its Beef Cattle Research Section. Initially the appointee will work at the headquarters of the Division at the Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., where there are active groups in the reproduction, nutrition and endocrine fields. The final location of the appointee will be in Queensland. CSIRO already has several research teams working in north-Eastern Australia. The biological problems associated with the northern cattle industry are numerous and these provide a challenge for scientific research. Currently the Beef Cattle Research Section is closely associated with the Division of Tropical Pastures which has major laboratories in Brisbane and Townsville where good facilities exist for reproductive physiology investigations.

DUTIES A research scientist is required to investigate physiology of reproduction in beef cattle maintained in tropical and sub-tropical environments. In particular the appointee will investigate reproductive performance of beef cattle especially in relation to nutrition. He will not be required to work on infectious diseases of cattle but will be expected to maintain close contact with those working in this field.

QUALIFICATIONS A Ph.D. degree or postgraduate research experience of an equivalent standard and duration in the field of mammalian reproduction, supported by satisfactory evidence of research ability. Experience in the tropics and familiarity with beef cattle production are not pre-requisites.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. For an outstanding appointee a higher classification will be considered. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The duration of the appointment will be determined in consultation with the successful candidate; some applicants may prefer a fixed term appointment for three years. The appointment will be conditional upon passing a medical examination and an initial probationary period of twelve months may be specified. An indefinite appointment carries with it Commonwealth Superannuation Fund or Provident Account privileges.

Applications (quoting Appointment No.: 245/472), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64-78, Kingsway,
LONDON WC2B 6BD
by the **3rd July, 1970.**

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(2214)

UNIVERSITY OF BELFAST

TEMPORARY ASSISTANT LECTURESHIP IN ZOOLOGY

The Senate of The Queen's University of Belfast invites applications for a Temporary Assistant Lectureship in Zoology from 1st October 1970 to 30th September 1971. Applicants should have interests in aspects of invertebrate biology. The salary will be at an appropriate point on the scale £1,240 to £1,470 (under review), depending on the qualifications and experience of the successful candidate.

One copy of a letter of application, giving the names of two referees, should be sent to the Secretary, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland (from whom further particulars may be obtained) by 30th June 1970. (2265)

UNIVERSITY OF LEICESTER

DEPT. OF PSYCHOLOGY

S.R.C. RESEARCH ASSISTANT

Applications are invited to assist in a project on :

(1) Social behaviour and display in ducks.
(2) Colour vision in birds.

The appointment will be from August 15, 1970, and in the first instance runs for one year until September 30, 1971. Honours degree and some research experience expected. Salary scale up to lectureship level, depending on qualifications.

Applications, with curriculum vitae under names of three referees, should reach: Dr. U. Weidmann, Dept. of Psychology, University, Leicester, before June 14. (2240)

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

SENIOR APPOINTMENTS IN GEOGRAPHY

The Department of Geography proposes to make two appointments during 1970. One of these will be to a

CHAIR OF GEOGRAPHY

and the other to a

LECTURESHIP OR SENIOR LECTURESHIP IN GEOGRAPHY

One of these vacancies is a newly-created post, the other a replacement appointment. The new professor will fill the second chair in the Department, and it is envisaged that he will be, for a period of time, Head of the Department, alternating in this position with the present Head of the Department, Professor Gilbert J. Butland.

The principal academic needs of the Department, which it is hoped the new appointees will fill, are in the fields of Geomorphology, Regional Planning and South-East Asian Regional Geography, but of greater importance than these specific specialisms is the academic excellence of the applicants, no matter what their special interests are, as the appointees will be encouraged to develop teaching courses related to their research interests.

In addition, in view of the creation of the School of Natural Resources in the University, it is hoped that the Department of Geography will contribute to the development of the new School in the academic offerings of the two appointees in the fields of Hydrology and Regional Planning, in both of which the Department has already made a substantial beginning. It would be especially welcome, therefore, if the new Professor would build a strong, specialist team in one of these two directions, so that the Department's support of Natural Resources in aspects of environmental design, regional development, water resource utilisation and/or hydrogeomorphology would not only be significant but emphasize the applied nature of the geographical studies taught and researched within the Geography Department.

Preference will also be given in one of the appointments to applicants with field experience in South-East Asia, and with interest and competence in the construction of courses in the geography of that major world region.

Applications close on June 25, 1970.

LECTURER—DEPARTMENT OF BIOCHEMISTRY AND NUTRITION

The successful applicant may be required to teach Biochemistry to second year Rural Science and Science students and to third and Honours year Science students. First class facilities are available for research in most pure and applied fields. These facilities include a well-equipped radio tracer laboratory for hard and soft emitters, mass spectrometer, several gas liquid chromatographs with radio active monitoring system, spectrophotometers, automatic analysing equipment including amino acid analysers, infra-red spectrophotometers and a wide range of the usual laboratory equipment. The successful applicant would be expected to do independent research, either individually or in collaboration in studies at present being undertaken in the Department.

Applicants should have a Ph.D. degree and would be expected to take up their appointment early in 1971

Applications close on June 30, 1970.

Salary ranges: Professor, $A12,000 per annum.
Senior Lecturer, $A7,600 by $A230 to $A8,750 per annum.
Lecturer, $A5,400 by $A270 to $A7,020 by $A280 to $A7,300 per annum.

Conditions include provision for travel and removal expenses, superannuation, assistance in buying or building a home, and study leave. Study leave grants are available and credit may be granted for existing study leave entitlement.

Further information may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, WC1H 0PF.

(2279)

For further Classified advertisements see page x

THE POLYTECHNIC OF CENTRAL LONDON

Applications are invited for full-time posts as

RESEARCH ASSISTANTS in PHYSICS

duties to commence in September/October, 1970. The research will be either in the field of Vacuum Physics or Radiation Physics.

Candidates should possess a good Honours Degree in Physics. Students expecting to graduate this Summer are invited to apply.

Salary scale: £930 by £30 (2) to £990 per annum.

Applications should be made in writing to the Head of the Department of Mathematics and Physics, The Polytechnic of Central London, 309 Regent Street, London, W1R 8AL. (2225)

THE BRITISH COUNCIL

ARGENTINA

THE NATIONAL UNIVERSITY OF THE LITORAL, SANTA FE requires a PROFESSOR OF UNIT OPERATIONS (Food Processing) as soon as possible. Candidates men only, should have an M.Sc. degree from a university in Britain, teaching and research experience, and published work. A Ph.D. degree would be an advantage. A basic knowledge of Spanish on arrival at post is essential.

Salary: £2,850 to £3,750 per annum according to qualifications and experience. Overseas allowances within the following ranges: married (accompanied) £941 to £700, married (unaccompanied) £646 to £395, single £12 to nil. Children's and home education allowances. Income tax refunded if levied. Free furnished accommodation. Outfit grant. Employer's superannuation contribution paid. Medical and insurance schemes. Fares paid for Professor and family, including mid-tour passages for children in Britain. Two-year contract guaranteed by the British Council.

Write, quoting reference number 9/OU94 to Appointments Division, The British Council, 65 Davies Street, London W1Y 2AA, for further particulars and application form to be returned completed as soon as possible. (2226)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

Applications are invited for the post of RESEARCH FELLOW or RESEARCH ASSISTANT in the above Department to work on hooded crows in West Scotland. Duties will be to initiate and develop a study of the feeding ecology of hooded crows, and more specifically, their relationship to hill sheep; and to co-operate in a population study. Applicants should be graduates in a biological subject with field experience of birds or mammals and a good understanding of ecological processes. The successful candidate will be expected to work from Oban, Argyll and ability to drive is essential. The post is tenable from 1st October, 1970, for three years.

Salary will be in the range £1,000 to £1,600 per annum depending on qualifications and experience, together with F.S.S.U.

Applications (two copies), giving the names of two referees, should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL. Please quote reference 5020. (2266)

HOUGHTON POULTRY RESEARCH STATION, HOUGHTON, HUNTINGDON

(Grant-aided by the Agricultural Research Council)

Applications are invited for the following posts in the grades of

SCIENTIFIC OFFICER OR SENIOR SCIENTIFIC OFFICER

(i) ELECTRON MICROSCOPIST

in the Leukosis Experimental Unit

Applications are invited from medical, veterinary or science graduates with experience or an interest in electron microscopy. The successful candidate would join a group studying avian virus tumours with particular emphasis on Marek's disease, and would be responsible for a small electron microscope unit. The work would include ultrastructural characterization of the lesions of Marek's disease and collaborative studies designed to develop techniques for the concentration and purification of Marek's disease virus.

(ii) VIROLOGIST

in the Virology Department

to assist in an investigation of avian respiratory infections. The work will entail studies on the incidence of respiratory disease in poultry flocks and investigations into the characteristics of viruses found to be involved.

Veterinary qualifications desirable but not essential.

Excellent facilities in both posts for experimental work and opportunities to work for a higher degree.

The appointment will be made in the scientific officer or senior scientific officer grade according to age, qualifications and experience. Salary scale: £1,162 to £1,982 or £2,193 to £2,703. F.S.S.U. superannuation.

Further particulars and application forms from the Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, to whom completed application forms should be returned by July 17, 1970. (2256)

UGANDA

TECHNICAL OFFICER

required by the Medical Research Council for their Child Nutrition Research Unit, Kampala, Uganda.

The Unit is studying nutrition and its disorders in infancy and childhood by several means, especially biochemical. The Unit consists of a metabolic ward, a series of laboratories containing modern, automated equipment, a newly built primate house and out-patient clinics. The successful candidate would be expected to become responsible for the general running of the primate house. He would preferably have experience in microbiology and in routine biochemistry. The qualification required is Higher National Certificate or its equivalent.

This post is newly created and is immediately available.

Appointment initially for a tour of 30 months at a salary on scale rising to £2,192, plus overseas allowances of up to £1,125 (married) and £570 (single). Education allowances and two free passages a year for children at U.K. Boarding Schools. Contributory Superannuation. Free furnished accommodation. Full details of conditions of service will be given at interview.

For further information and an application form please write to Mr. E. P. Aboud, Personnel (Overseas), Medical Research Council, 20 Park Crescent, London W1N 4AL, quoting N240/260.

(2230)

ETHICAL PHARMA

One of the world's leading ethical pharmaceutical manufacturers, wit fine record of research over more than 40 years, wishes to make the following important appointments at its headquarters in the Netherlands. The reputation of the company has been built on worldw acceptance of its range of ethical products, originated by research an development teams with a total staff of more than 600. These scientis are drawn from many countries, including a number from the U.K.

SENIOR PATHOLOGIST

c£5,500

A Senior Pathologist is required to take charge of the histopathology laboratory which performs an important part of the company's drug safety evaluation work.

A first-class background in veterinary or medical pathology is required, preferably with experience gained during toxicity trials.

The department deals with a high volume of work, and candidates must be experienced in lucid report writing and data presentation. The department is well staffed at both scientific and technical levels.

Age is not important, but candidates must have appropriate postgraduate qualifications as well as substantial experience.

SENIOR PHARMACOLOGIST

c£5,000

CARDIOVASCULAR RESEARCH

The Department of Pharmacology has primary interests in the cardio vascular, anti-inflammatory and central nervous system fields, and the future development of drug research in these areas requires the appointment of two additional Pharmacologists.

The department is exceptionally well equipped and backed by experienced supporting staff. Collaboration with other research disciplines is close, especially with the department of Basic Human Research, which offers opportunities for clinical contacts. Cordial relations are maintained with a number of important university departments of pharmacology, and publication of results and attendance at scientific meetings is actively encouraged.

The Senior Pharmacologist will take charge of the cardiovascular group and post-doctoral experience in the analysis of drug action in this field would be a definite advantage.

The successful candidate is likely to be in his early 30s and will have probably graduated in medicine, physiology or pharmacy. His postgraduate experience will have led to the award of a PhD, M.D. or equivalent in pharmacology. This appointment will appeal to a young but mature pharmacologist, whose record of achievement shows that he has the professional capability, energy and enthusiasm to lead a specialist group.

PHARMACOLOGI

c£4,000

NEURO-PHARMACOLOGICAL RESEARCH

This appointment will appeal to a Pharmacologist with a basic intere the functioning of the central nerv system. He should be capable of initiating and maintaining a researc programme within a group devote the exploration of new approaches therapy of nervous dysfunction.

The successful candidate, who is li to be in his late 20s, or early 30s, m have graduated in medicine, physi or pharmacy, and will have comple postgraduate studies to at least Ph MD level, preferably in neurophar cological or physiological research.

CONDITIONS OF SERVICE. The company's salary policy allows for ample recognition of merit and conditions of service and fringe benefits are excellent.

TRAINING AND DEVELOPMENT. The company offers training and development opportunities to all its staff, and encouragement is given for attendance at scientific meetings, and for publication of results.

LANGUAGES. English is freely spoken by all management and scientific staff, and additional facilities are available for learning other European languages.

LOCATION. All these appointments are located at the company's headquarters, which provides a pleasant country town environment with good housing, education and recreational facilities. The company will provide every assistance with removal and relocation.

NATURE

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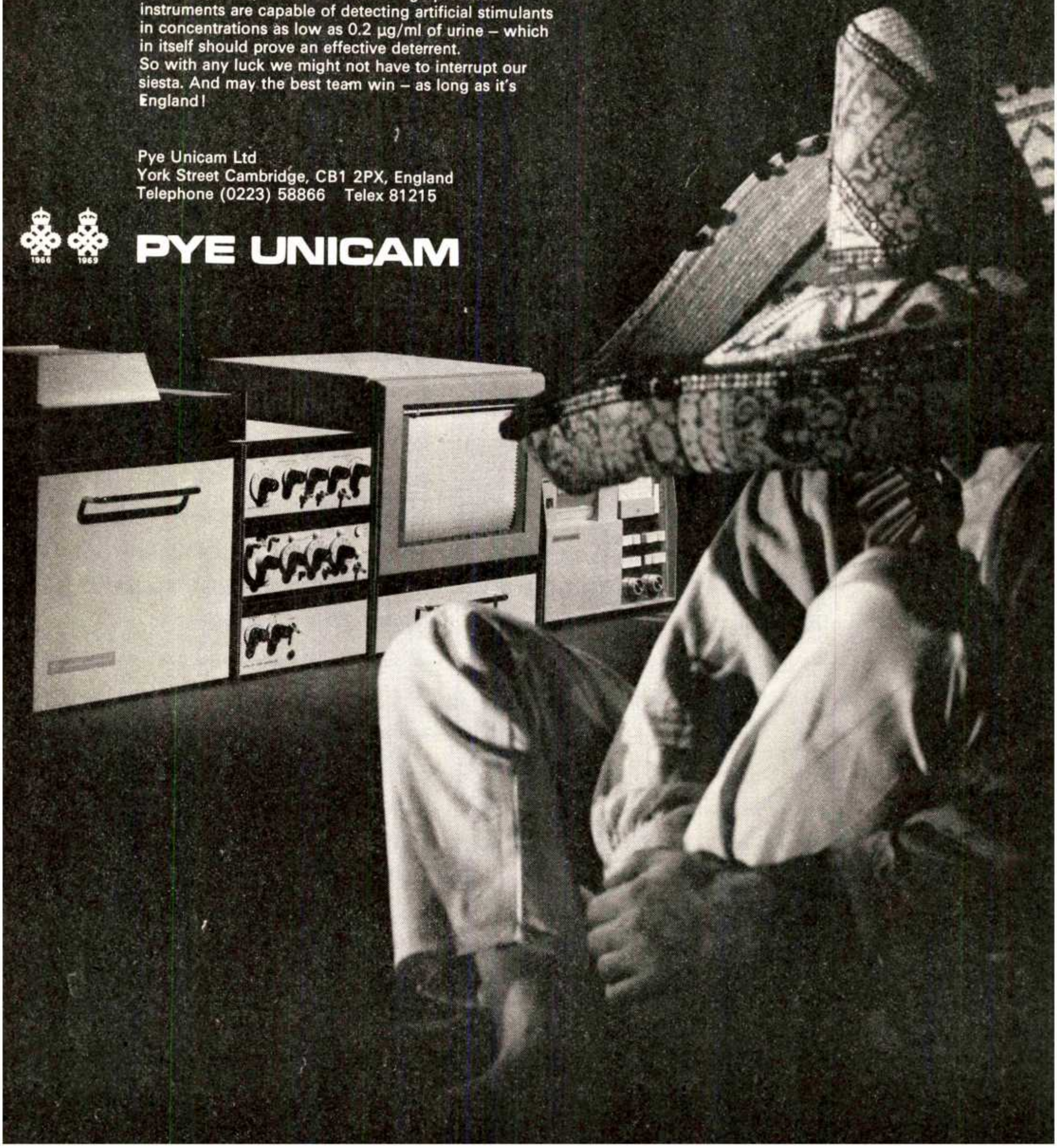

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Keeping on the Straight and Narrow Path

EVERYBODY is anxious to give the Food and Drug Administration a fair chance to prove that it can rise like a phoenix from the ashes of its old reputation, and there is every sign that the new Commissioner, Dr Charles Edwards, is beginning to make a mark. This is why it would be wrong to make too much of the way in which Mr Fountain's committee last week seems to have uncovered an unhappy tendency towards irregularity in what is, after all, one of the most important—and certainly the most newsy—of the regulatory agencies in the United States. The cyclamate business, after all, has been an unseemly business from the start. To be sure, it will at this stage be something of a comfort for Dr Edwards and his colleagues that there seems to be no substantial case for saying that the FDA has been concealing—or hiding from—evidence that cyclamates are dangerous since the beginning of the sixties. With the worst will in the world, the most that can be said is that it might have been justifiable to take them off the market a year earlier, towards the end of 1968.

Given that the most serious argument against the use of cyclamates is that their efficacy as aids to weight reduction is still in doubt, the most ungainly feature of the pantomime proceedings on October 18 last year is that the banishment of cyclamates was undertaken in such unseemly haste. Can it ever be good government that an important decision affecting not merely the propriety of manufacturers but the peace of mind of a large part of a very large population should be made on a Friday afternoon and made public on the Saturday? Would it not have been much better to have waited for time to plan an announcement that would seem less a response to panic? And would there not have been a case for waiting for more convincing experimental results than those on which the first decision was taken? With this dirty water under the bridge, nobody should be surprised that there should have been attempts to go back on the original decision. That the FDA should have allowed outsiders—even powerful outsiders—to use its machinery for doing this is reprehensible even if, this once, forgivable.

The way in which the FDA should seek to deal with the safety of drugs and food is one of the questions provoked by the inquiry last week, and there is very little doubt that Dr Edwards is right in saying that the first task is to restore a sense of pride to a battered agency. So far, so good. It is also important that some method should be found for assessing more objectively the biological hazards of new chemicals. But the hazards on which attention is now concentrated are not outright poisons but, rather, materials whose effects are necessarily covert and slow. Moreover, in the assessment of the risk of using this or that chemical, there is bound to be a period when experimental evidence first accumulates when it is exceedingly hard to interpret this evidence. This, for practical purposes, is the reason given by the FDA for not acting sooner on cyclamates. It would have been quite proper to wait for even longer. As time goes on, it will be increasingly important to decide whether some material not previously in use should be licensed as safe, which should provide only minor problems. Yet there are bound always to be materials, not necessarily food additives, which are already in use when their safety is questioned. The problem is what to do in the period before such a suspicion can be fully tested. It would be a great boon for the public peace of mind if the FDA were now publicly to resolve to take a measured view of the evidence, circumstantial and otherwise.

What does this imply for the Delaney amendment, the form of words by means of which Congress has sought to put an end to cancer? Dr Edwards last week nailed his colours to the mast of this legislation, but that is hardly the point. The practical question is how the amendment is to be applied. What kind of evidence is to be taken as a proof that some foodstuffs may be carcinogenic? Is there to be a ban the instant that a tumour appears in an experimental animal? What does Congress require for a test of significance in the comparison between a group of experimental animals and the controls? Is a probability of chance occurrence less than 0·5 sufficient to outlaw some previously unsuspected chemical? Or will Congress wait until the evidence justifies a more certain conclusion? By cheering on the Delaney lobby last week without raising the questions which should still have been fresh in his mind about the cyclamate decision bequeathed to him by Dr Herbert Ley, Dr Edwards may find that he has given an uncomfortable number of hostages to fortune.

Another difficulty in the administration of this legislation is the means by which the FDA should combine its judicial and its scientific functions. Mr Fountain's committee may too lightly have ignored last week the value of the research which the FDA carries out, but nobody seems fully to recognize the intellectual difficulty of the judicial function. If there is anything at all to be learned from the unseemly taking of decisions on cyclamates and on the package insert for oral contraceptives, it is that the quasi-judicial proceedings of the FDA should be made more formal. Other regulatory agencies of the Federal Government have several commissioners to share responsibility and it is hard to see why the FDA should not move at least a good distance in that direction by the appointment of an old-fashioned council to solemnize decisions and to share responsibility for them. Without such an instrument, properly constituted by Congress, each successive commissioner will find himself intolerably squeezed between the bureaucracy of HEW and the imitators of Mr Ralph Nader.

The FDA also needs a more rational definition of the function of its scientific work, the origins of which are to be found in the simple days when the acme of vigilance was the identification of unexpected contaminants in ordinary food—insect parts in flour was a perennial favourite. The task, in short, was the enforcement of regulations already in force. The novelty in the past few years has been the way in which the FDA has also assumed some of the public responsibility for seeking out those agents in the environment, natural as well as artificial, which may be suspect. Dr Henry Simmons, the director of the FDA's Bureau of Drugs, put one of the obvious difficulties well when he described last week the way in which turning over every stone raised a host of unexpected problems. The trouble, of course, is that, however hard it tries, the FDA will never be able to do everything that might be tried. Some of its attempts to outdo Mr Nader in the past few months have shown that, with the best will in the world, the FDA's scientific branch will be able only to tackle a small part of what needs to be done if mutagenesis and teratogenesis as well as more tests are to be applied to the ingredients of ordinary life.

But if the FDA cannot be at once judge and detective agency, where should it concentrate ? The most urgent need, of course, is that the FDA should be strongest in the determination of the criteria on which safety and danger are determined. What this implies is that the FDA should be skilled at defining the protocols which must be satisfied by the tests which manufacturers carry out, and that it should be most of all concerned to advance the techniques by means of which these tests can be applied. At the same time, it should be wary of trying to function as a fire-fighter whenever the prospect of some new danger is raised—the danger is that too rapid investigation with inadequate resources may serve only to confuse the issue (as with cyclamates). In the long run, the FDA's best hope is that by the judicious placing of contracts with outside researchers, and by the stimulation of a professional interest in this important work, the FDA can more effectively harness the energies of the scientific world to its important cause. In doing this, however, the FDA would be well advised to lessen its growing dependence on the committees of the National Academy of Sciences—the objective should be a vigorous and open community of active researchers and not a device for investing decisions with prestige.

There remains the most glaring and the least readily acknowledged of all the FDA's shortcomings—its tendency to act independently of what happens elsewhere. In this sense, the United States is well on the way to becoming the largest island in the world. But is there any reason to believe that manufactured chemicals such as 2,4,5-T are different in the United States and Europe ? Is there no means by which the assessment of the hazards of drugs, food additives and other potentially hazardous materials may be carried out internationally ? It is true that arrangements for such activities might seem to be a diminution of the right of Congress to look after the health of United States citizens, but that is not a serious consideration. If Dr Edwards wishes to make a constructive mark on the FDA, he will seek not merely independence from the secretary of HEW but also a much closer working relationship with comparable organizations elsewhere.

A Cure for Cancer Research

THE appointment of a committee to coordinate cancer research in Britain, many years overdue as it is, is one of the most hopeful signs of progress in this inevitably troubled field. The delay in appointing the committee, once the Medical Research Council had decided to set it up more than six months ago, aroused fears that the committee was not intended to play any serious part in cancer research. But now that the members have at last been announced, it seems that the committee should be well able to exert a firm and beneficial management over its field of influence.

The committee, which is to be known as the Coordinating Committee on Cancer Research, is sponsored by the three organizations that fund cancer research in Britain. These are the Medical Research Council (MRC), the Cancer Research Campaign (CRC), which recently dropped the British Empire from its erstwhile title of the British Empire Campaign for Cancer Research, and the Imperial Cancer Research Fund (ICRF).

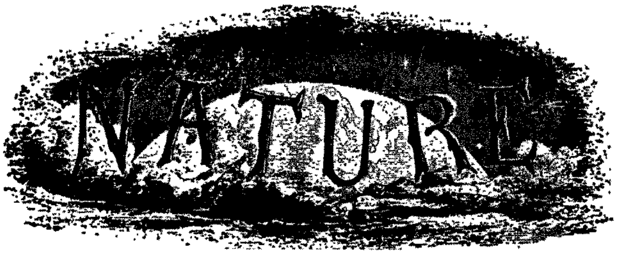
Membership of the committee is restricted to the research directors of the four major British institutes—Dr M. G. P. Stoker of the ICRF, Professor T. Symington of the Institute of Cancer Research, Dr L. G. Lajtha of the Paterson Laboratories, Christie Hospital and Holt Radium Institute, and Dr John Paul of the Beatson Institute for Cancer Research, together with representatives of the three funding agencies, Sir Brian Windeyer for the MRC, Professor T. Crawford for the CRC and Professor E. F. Scowen of the ICRF. The chairman is Sir Max Rosenheim of University College Hospital Medical School.

The chief use of the committee will be as a forum where the directors of research can talk to each other with the keepers of the purse strings listening in. There is much to talk about. For lack of any previous attempt at coordination, it will be a sizable task simply to catalogue who is doing what and why. Then will come the real work of the committee, that of trying to assess the relevance of this work to cancer research, and whether the present distribution of money and manpower is reasonable. In other words, the committee's decisions will affect not only the domestic policies of the four institutes concerned but also the broad issues of national policy such as whether screening of anti-cancer drugs or experimental chemotherapy should be concentrated at a single institute or dispersed among several centres.

Although the three sponsoring bodies have stated that the committee's function will be purely advisory, it would be tragic if their representatives on the committee should fail to ensure that its advice is listened to. Between them the MRC, the CRC and the ICRF dispose of some £4 million a year on cancer research, which accounts for a sizable fraction of Britain's research expenditure and which, because of the breadth of cancer research, has a significant effect on medical and biological research in general. With the appointment of the committee there is at last an opportunity for devising a coherent strategy as to how these funds should be deployed.

The committee is to hold its first meeting early next month and if all goes well it will probably continue to meet at frequent intervals. One of its first decisions will be how far to make public its judgments and it will be a good augury for an innovative future if it elects to depart, even a little, from the standards of secrecy practised by government bodies such as the MRC.

100 Years Ago



LETTERS TO THE EDITOR

[*The Editor does not hold himself responsible for opinions expressed by his Correspondents. No notice is taken of anonymous communications.*]

The Corona

Having been informed that my remarks at the meeting of the Royal Astronomical Society, on Friday last, have been interpreted otherwise than I meant, doubtless in consequence of my having spoken without preparation, I beg to repeat what I intended should have been the purport of my statement.

It seems to me beyond reasonable doubt that we have upon all the photographs, whether of long or of short exposure, a representative of *something which is at the sun*, since the contour of the radiance depicted upon all the photographs exhibits minima, in directions closely approximate to the extremities of the sun's axis of rotation. Furthermore, exterior to all this, and apparently masking it in a very great degree to the ordinary observer, is a much wider and more conspicuous radiance of very irregular outline, to which the name *Corona* has been ordinarily applied. The streamers and irregular projections of this latter corona appeared to me to vary in position during the period of totality in August last. They seem, moreover, to have no connection with the positions of the prominences, nor yet with that of the solar axis, and hence I infer that this phenomenon arises from something *which is not at the sun*.

London, June 15 B. A. Gould

Gould, who was born in Boston, is best known for his catalogue of southern hemisphere stars. *From* Nature, **2**, 141, *June* 23, 1870.

OLD WORLD

ELDO

Successful Failure for Europa 1

Once again the Europa 1 rocket of the European Launcher Development Organization has failed to put a 260 kg Italian test satellite into orbit. This was the sad news from Woomera on June 12 after the last test of the rocket before the programme moves to the new launching site in French Guiana. ELDO has now marked up an immaculately complete history of failures stretching back to August 1966, an inauspicious record to lay before the forthcoming European Space Conference at Brussels.

What seems to have happened this time is that the nose fairings which protect the satellite during the flight through the lower atmosphere were not jettisoned, so that the weight of the payload was increased to an unacceptable value. According to the flight plan the fairings should have been released 223 seconds after launch during the firing of the second stage engines, at a height of about 150 km. That this did not occur was apparently due to the premature disconnexion of a connector plug 78 seconds into the flight. The fairings added something like 300 kg to the weight of the payload.

ELDO is used to putting a brave face on things, and this week a spokesman described the flight as "on the whole very satisfactory". It is true that by ELDO standards the firing could have been called a roaring success. For the first time all three stages of the multinational rocket functioned correctly so that the problems which caused the German third stage to explode on the previous two flights seem to have been solved. According to an article in *Le Monde*, it could have been that the autodestruction mechanism in the German stage was being triggered spontaneously by stray potentials. This would have given rise to the sort of rapid increases in pressure in the fuel tanks that were recorded during the previous two flights. Therefore the most important objective of the flight was achieved, and on the face of it the non-release of the nose fairings ought to be easily solved.

Whether this will cut any ice at the European Space Conference remains to be seen. Postponed to July 22–24 because of the British elections, the conference is at ministerial level and is expected to make at least a start on setting the future pattern of European work in space. In the first place it is hoped that the conference will take a further step towards rationalization of the European space effort by combining ELDO with its sister agency, the European Space Research Organization. But behind all discussions about the part that Europe ought to be taking in space is the debate over whether Europe needs the luxury of a launcher of its own, and the past history of ELDO is inevitably colouring the argument.

Had everything worked perfectly, Europa 1 would have orbited an Italian test satellite of 214 kg and an adaptor carrying radio guidance equipment. Other aims of the flight were to carry out communications experiments using equipment supplied by Belgium, Denmark, Italy, Norway and Sweden and installed in the satellite, and to test the operation of the ground stations associated with the ELDO programme. But this was also the last flight of Europa 1. In the ELDO scheme of things the next step is to test the second configuration of the launcher which includes a French perigee motor on top of the German stage. Eventually everyone hopes that this combination will launch the Franco-German communications satellite.

SOVIET UNION

Madness in the Method

from our Soviet Correspondent

THE case of the geneticist Zhores Medvedev, at present held in a Kaluga mental hospital because of his open protests on the "senseless barriers" imposed by the Soviet authorities on academic and scientific freedom (*Nature*, **226**, 887; 1970), is unfortunately not unique even in the recent annals of Soviet science. The use of the psychiatric hospital as an alternative to prison or Siberian exile was first inaugurated under Stalin and has been resorted to on many occasions since. Among the most noteworthy cases of the last few years are the following.

N. I. Samsonov, a geophysicist, who was awarded a Stalin prize for his arctic research, was interned in the Leningrad psychiatric hospital from 1956 to 1964, after writing to the Central Committee of the CPSU criticizing some of Stalin's theoretical propositions.

Yu. V. Levin, a biologist, wrote to the United States embassy in Moscow deploring the Soviet intervention in Czechoslovakia; arrested and sent to Leningrad psychiatric hospital in November 1968, he was discharged after ten days as perfectly sane.

Petr Grigorenko, now most widely known under his military rank of major-general, was for 17 years director of research and later the head of the cybernetics department of Frunze Academy. He was arrested and declared insane in 1969 for his support of the displaced Crimean Tartars.

Vladimir Borison of Leningrad, a philologist, is now in the Leningrad psychiatric hospital; according to one underground source, his doctors say, "He's perfectly normal, of course, but orders are orders".

Perhaps the best known case is that of the mathematical logician, Aleksandr S. Esenin-Volpin, who since 1949 has been confined to a mental hospital on some five separate occasions. Such work as he has been allowed to publish in interludes of freedom shows no very evident sign of mental disturbance. His most recent arrest, on May 5, 1968, evoked a wave of protests, one of which was signed by 99 mathematicians and physicists throughout the Soviet Union (15 of whom later withdrew their signatures). Since this last arrest, Esenin-Volpin has been subjected to heavy police surveillance, and is not allowed to hold any university post. He is working at present in the All-Union Institute of Scientific and Technical Information—a somewhat routine post, much below his intellectual capacity.

Rehabilitation of erstwhile dissidents has been a notable feature of Soviet policy changes, and some scholars and intellectuals have been fortunate enough to be rehabilitated in their own lifetime. It is, perhaps, one of the more ironic aspects of the Medvedev affair that, almost simultaneously with his arrest, an article appeared in *Soviet Statehood and Law* ((5), 1970), which recommends that special provisions for the liability of the officials concerned in the case of such illegal dismissals or transfers should be included either in a special labour act or in the basic principles of the labour law of the Soviet Union and the Union republics.

DOCTORS' PAY

Consultants and Professors

THE British Medical Association has at last decided to exchange the public prestige of the medical profession for the freedom to bargain like a trade union. In many ways this is a sensible decision because the prestige was being eroded anyway, and the astonishment occasioned by the avidity of the BMA's demands will soon be forgotten. But how will the pay awards to doctors affect people in the universities? Doctors are, after all, only applied biologists, and their training is not necessarily more protracted than that of many academic scientists.

One point at which the recommendations may immediately create ill-feeling is that staff in medical schools who hold honorary NHS contracts, and are paid according to the NHS consultants' scale, will receive a 30 per cent salary increase that will create discrepancies in university pay scales. All medical staff who hold NHS contracts would be paid more than senior lecturers in other departments, and the average professor would be paid less than the average consultant in the medical schools. The old consultants' scale stretched from the middle of the scale for senior lecturers to slightly above the average for professors' salaries. The Association of University Teachers is holding its fire until the Prices and Incomes Board has given its verdict on the recommendations made by the Kindersley committee.

The council meeting of the British Medical Association, reported in last week's *British Medical Journal*, is an excellent example of a learned profession standing on its badly dented dignity. "Our sense of justice has been violated," declared the chairman, Dr Ronald Gibson, and "it is a national disgrace that a noble and dedicated profession should be manhandled and insulted in this way". It is obvious from the report of the meeting that the BMA was never in doubt about the justification for taking industrial action over the reference of half the Kindersley recommendation to the Prices and Incomes Board, and the real question was how far out on a limb doctors could reasonably go. Amidst

Table 1. UNIVERSITY SALARIES AND KINDERSLEY RECOMMENDATIONS FOR DOCTORS' SALARIES

University salaries:	
Assistant lecturers and lecturers	£1,352 to £3,097
Senior lecturers and readers	A range of scales with maximum of £3,900
Professors	Minimum £4,019. The average at any institution not to exceed £5,096
Kindersley recommendations for doctors' salaries:	
House officer	£1,626 to £1,884
Senior house officer	£2,040 to £2,328
Registrar	£2,328 to £2,886
Senior registrar	£2,760 to £3,588
Consultant	£4,512 to £6,855

(The article in *Nature*, **226**, 993; 1970, should have made clear that the figures quoted for doctors' salaries represented maximum scales.)

the high flown oratory which the meeting produced, there was a clear call for resignation as a matter of principle. Many doctors present at the meeting thought that the profession would regard the decision to stop signing medical certificates and to refuse to participate in NHS committees as not going far enough, and Mr J. Elkington, chairman of the BMA's Hospital Junior Staffs Group Council, declared that resignation only meant resignation from the health service, and that doctors would go on looking after patients even if they worked for nothing and even if they starved. It will be interesting to see whether the referendum being conducted by the BMA will bear out the council's estimate of the wrath of the medical profession. Last weekend, the BMA sent about 50,000 forms to doctors, asking whether they would resign from the health service if the review body is not reinstated.

If the level of support for the BMA's recommendation that doctors should refuse to sign sickness certificates is any yardstick, then the council might be in for a shock. To be sure, the BMA claims that its sanctions are enjoying 100 per cent support in some areas, but the Department of Health and Social Security puts the figure at more like 75 per cent, and it seems that several doctors who originally refused to sign certificates have since changed their minds. There is also a clear call from the *Lancet* that the BMA should moderate its temper, and that "thoughts of organized resignation from the health service be banished".

What, then, are the chances of the doctors' militancy getting out of hand ? One factor is that the effect of industrial action will be much less after the election than it was in the middle of the election campaign. In any case, there seems to be a substantial body of opinion even inside the medical profession that doctors are being greedy in sticking out for the full 30 per cent increase. As one consultant said in a letter to *The Guardian*, "I have been amazed at the irresponsible recommendations of the Kindersley committee: well might they resign."

FISHERIES

Sanity on Salmon

Limitations have at last been imposed on salmon fishing in the Greenland area, and in comparison with the restrictions agreed last month for the north-east Atlantic they are surprisingly strict. The prolonged international wrangle about alleged overfishing by Scandinavian drifters was resolved in Newfoundland this month at the annual meeting of the International Commission for North-West Atlantic Fisheries (ICNAF), which decided that each member country should prevent either the tonnage of its salmon fleet or its annual catch in the north-west Atlantic from exceeding the 1969 size. This restriction covers all salmon fishing outside the three-mile limit around the coast of Greenland.

Because ICNAF cannot, according to its constitution, simply impose a maximum tonnage on a national fleet, the individual countries have been left with a choice, but the important point is that limits of one kind or another have been agreed. Several countries, including Britain, were dissatisfied when the counterpart body of ICNAF, the North-East Atlantic Fisheries Commission (NEAFCO), decided last month to restrict salmon fishing on the European side of the Atlantic to an eight-week season each year but not to stop the fleets from expanding (see *Nature*, **226**, 678; 1970). The difficulty then was that any stronger proposal would have been outvoted by Denmark, Sweden and West Germany, and the group of countries, led by Britain and Canada, which had wanted a complete ban to protect their own stocks of salmon, had to be content with rather less than half a loaf.

The restrictions which were eventually agreed upon at the ICNAF meeting were in part the outcome of discussions between English and Danish representatives in the period since the NEAFCO meeting. In the final vote, the Danes, Swedes and West Germans came out in favour of keeping the catches down, and also approved of restrictions on the size of nets that can be used and on the length of the fishing season. Salmon are now to be fished only from August to November; this makes little difference to the principal fishery off Greenland, because the fish are not there at any other time of the year, but will stop the industry from making further inroads into the Davis Strait between Greenland and Canada, where salmon are around for somewhat longer.

Britain is, on the whole, happy with the decision, for in spite of the lack of clear evidence so far, it had been feared that British stocks of salmon might fall if the exploitation of the salmon's Atlantic breeding grounds continue to grow without check. The ideal remedy, that of banning the fishing of salmon on the high seas altogether, would never have been agreed to by the ICNAF meeting.

CONSERVATION

No More Blackbird Pie

Small birds will soon be able to fly fearlessly across the borders of Italy, for the Italian Minister of Agriculture, Mr Lorenzo Natali, intends to introduce a new law to forbid bird netting, a form of hunting that can account for the death of some 10 million migrant birds each year.

This decision, announced by the Italian delegation to the Council of Europe, is surprising because it was only in January this year that the Italian government gave way to one of its most powerful lobbies—there are about 1,800,000 hunters in Italy—and passed a Bill permitting the netting of small birds, thereby negating a Bill passed some three years earlier which had stopped netting in most of its aspects. The government argued that the new Bill was necessary to enable birds to be captured for scientific research—a claim strenuously denied by the Italian National Research Council—but it was clear that most of the netted birds would end up not in a laboratory but in pies or pâtés.

The Bill evoked an immediate outcry all over Europe; it seemed especially tactless that the Bill should be passed in the first month of European Conservation Year. Conservation societies voiced their complete opposition and many sent protests to the Italian government. The International Council for Bird Preservation, for example, urged people to write to Italian embassies and tourist boards to make their views known and the response to this plea was apparently world-wide. In Italy there was a strong body of opinion opposed to the Bill, and Dr P. L. Florio, senior trustee of the Italian National Appeal of the World Wildlife Fund, had collected half a million signatures on a petition which was to be presented to the government in the near future. In March the World Wildlife Fund sent a request to the Italian Foreign Ministry and the Ministry of Agriculture asking for the total abolition of bird netting in Italy.

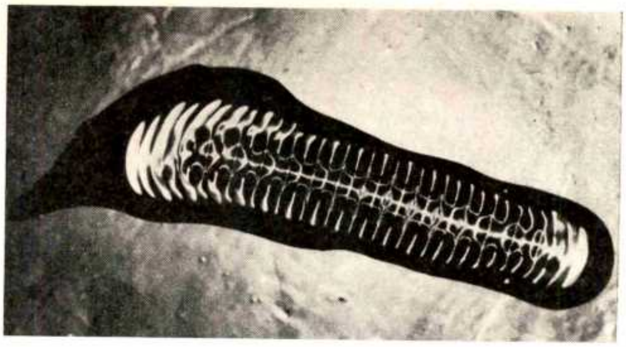
It seems that all this pressure has borne fruit and the Council of Europe now hopes that other countries, such as France, Greece, Spain and Portugal, will soon follow Italy's example. It is hard to know how widespread netting is outside Italy, but statements to the council from national delegations and from the International Hunting Council have revealed a thriving trade in netted birds between several member countries, and the council believes that coordinated legislation is needed in all parts of Europe against netting and the importing and exporting of netted birds.

EXHIBITIONS

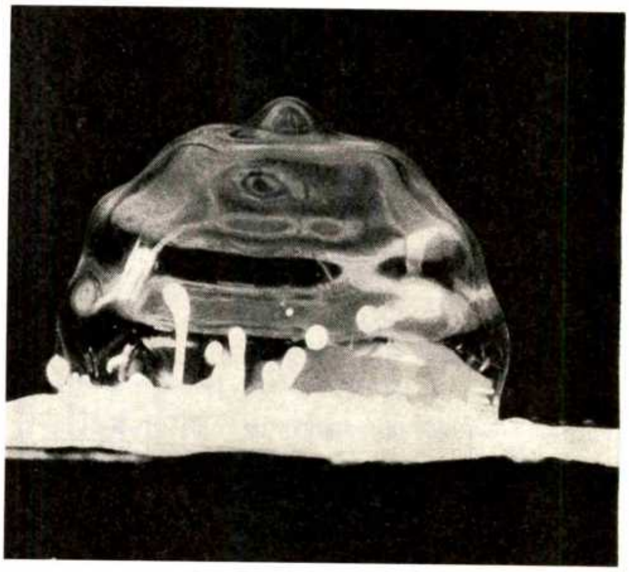
Patterns of Sound

A REMARKABLE exhibition of matter tortured by sound is on display until July 22 at the Institute of Contemporary Arts in London. The exhibition is the work of Dr Hans Jenny, a Swiss physician, who has improved on Chladni's contribution to science by extending the range of forms and shapes that can be extracted from matter by vibrations. Whereas Chladni to get his famous figures merely sawed with a violin bow on a sand-strewn metal plate, Dr Jenny has applied crystal oscillators to glycerine, mercury, lycopodium powder, turpentine and the many other substances that are grist to his mill.

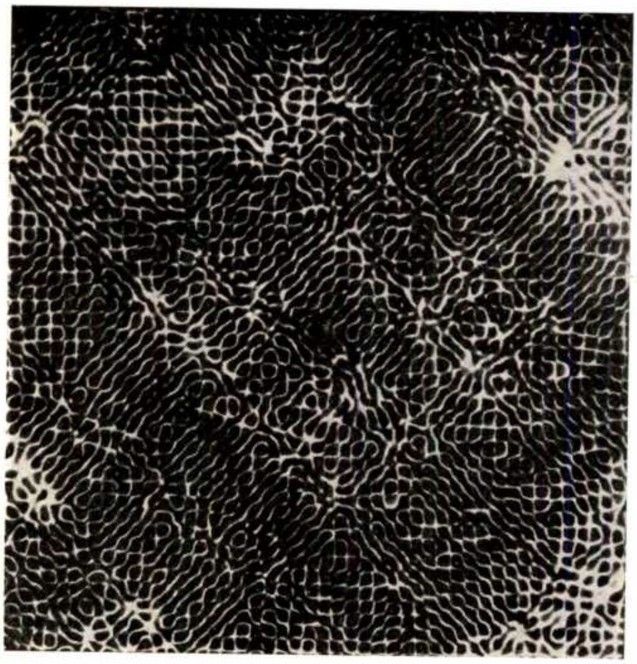
The results, recorded in the form of photographs and a non-stop film show, are a spectacular travelogue to a through-the-mirror land where ordinary physical laws seem to be held in abeyance. Who would believe that a soap bubble could shape itself into a dodecahedron and grow a stalk, looking to all the world like a bacteriophage ? Or that water droplets could move sideways in squares ? Or a blob of glycerine metamorphose into a vertebral column with 35 segments ? Jenny's work shows how inanimate matter charged with vibrational energy can assume shapes of unexpected subtlety and vitality. Anyone who is tired of the humdrum behaviour of matter in the laboratory should visit the ICA exhibition to see the elegant but other-world sculptures that can be conjured out of paste, powder and iron filings.



Glycerine vibrated at 30 Hz (length of figure is 4 cm).



Soap bubble vibrated at 110 Hz forming a pulsating polyhedron (spikes of liquid soap in foreground).



Chladni figure formed by quartz sand vibrated on a steel plate at 16,800 Hz.

UNIVERSITIES

Imperial Quart in Pint Pot

THE Herculean task of reforming the administrative structure of London University with its three-quarters of a million students and eleven federated colleges has been allotted to an independent committee under the chairmanship of Lord Murray of Newhaven, the director of the Leverhulme Trust Fund. Following the recommendations made by the university senate last January (see *Nature*, **225**, 492; 1970) the chief aim of the committee will be to look into the future of London University as a federal institution, concentrating particularly on such questions as the function of the senate, the relation between individual colleges and the university, and student participation.

The committee has been appointed jointly by London University and the University Grants Committee (UGC) and the hope is that it will report within two to three years. Five of the ten members are from outside the university and include Professor W. M. Cooper and Professor F. S. Dainton, the Vice-Chancellors of Manchester and Nottingham Universities respectively.

One of the most immediate problems before the committee is what to do about the 35,000 external degree students who are examined but not taught by the university. Despite the establishment of the Council for National Academic Awards (CNAA) in 1962, the number of external students has been growing rapidly and the senate has warned that if the number of school leavers increases as fast as the Department of Education and Science predicts, the present London University external degree system will be unable to cope.

Some of the hardest in-fighting may be done over the distribution of finances within the university. The University Court is responsible for dividing out the block grant from the UGC between the individual colleges although for the present, at least, Imperial College and Chelsea College have funds allotted specially for them by the UGC. But rumours of secession and unilateral declarations of independence have long been in the air, and up and down Prince Consort Road and in the shadows of the Albert Hall there can be heard faint mutterings that Imperial College would perhaps do better to be known to the world as the University of Kensington. This, no doubt, is one of the matters that will exercise the attention of Lord Murray's committee.

ATMOSPHERE

Whitewashing Coal

The development of fluidized bed processes for burning coal more economically and less poisonously, now under way in the laboratories of the National Coal Board, has attracted backing worth £125,000 from the US National Air Pollution Control Administration. According to an agreement signed this month by Lord Robens, chairman of the NCB, and Mr John T. Middleton, commissioner of NAPCA, the two organizations will jointly finance a £0·25 million programme which includes further research into the capacity of fluidized beds for retaining sulphur and oxides of nitrogen in the ash instead of liberating them into the atmosphere. This agreement follows a decision in principle to cooperate over the process, which was taken last year.

The basis of the process is that the air required for combustion is blown through a bed of coal particles, and because the solid mass then behaves as a liquid, boiler tubes can be immersed in the bed. As a result, heat transfer improves and the boiler need not be as voluminous as usual—a 660 MW boiler for a power station, for example, might be only about 50 feet high, compared with more than 200 feet for the most recent conventional designs, and if the coal is fluidized with air under pressure the size can be shrunk further. If limestone is added to the coal, most of the sulphur can be trapped in the bed, together with a fair proportion of the oxides of nitrogen.

So far plans for large fluidized bed boilers are confined to design studies, although the NCB is now thinking of installing a pilot unit at a power station in Grimethorpe. A small prototype shell boiler, giving 8,000 pounds/h of steam, has been running for more than a year at the Coal Research Establishment near Cheltenham, and this is providing the data on which larger designs are to be based.

DESALINATION

Freezing out Brine

Plans for a large experimental desalination plant to be built at Ipswich are being considered by the government, Lord Kennet, Parliamentary Secretary at the Ministry of Housing and Local Government, said in London last week. The proposals, put forward by the Atomic Energy Authority and the Water Resources Board, are for a plant to produce a million gallons of water a day using a new process in which the salt is removed by refrigeration.

The traditional means of desalinating water is by flash distillation, in which fresh water is obtained by repeated condensation. This method has been adopted by several countries which have embarked on desalination programmes, but its economic viability has often been questioned. The power consumed by the process is large, and the attractions of linking such an installation directly to a nuclear power plant have so far proved illusory. As pointed out by the head of the International Atomic Energy Agency at the recent conference on desalination in Madrid, only the advent of fast reactors will bring about a significant change in the economics of desalination by this means. But operational fast reactors are still a decade or so away.

For a country such as Britain, the relatively low temperature of the neighbouring seas is considered an additional factor in favour of experimenting with the refrigeration process. Less power is consumed in this process, in which impurities are removed in the form of a brine slurry by the addition of a hydrocarbon freezing agent, and although no large scale plant has yet been built, test installations built by Simon Engineering in Stockport—producing 10,000 gallons a day—and by the Atomic Energy Research Establishment, Harwell, have produced promising results.

When the desalination programme started at Harwell in 1965 the emphasis was chiefly on flash distillation. Over the first three years the government spent £1·3 million on this programme, and although there are more than 100 distillation plants for desalination in industry it became steadily clearer that for domestic supplies the refrigeration process was likely to prove more economic. Work on the desalination programme is also being carried out at the AEA establishments at Risley and Winfrith.

Although countries such as the United States and Israel are also interested in the refrigeration process, flash distillation is still the most common technique. The French company SIDEM is at present enlarging the desalination plant at Le Havre, which turns out a million gallons a day by distillation, and it is believed that for a country such as France with frequent shortages of water there will have to be increasing reliance on desalination plants in future.

NEW WORLD

Food and Drug Maladministration

THE Food and Drug Administration, which has been struggling since last December to find a secure basis for continued survival, fell flat on its face again last week at the public inquiry before Representative L. H. Fountain's subcommittee of the House Committee on Government Operations. Although the new Commissioner, Dr Charles Edwards, began his evidence by deliberately washing his hands of a good deal of the past, at the end of a gruelling two days he was reduced to silence by the chairman's declaration that the "integrity and importance" of the law on which the FDA's operations are founded is being "eroded" by the FDA's interpretation of it in the past few months. For a time, at least, it began to seem as if many of the charges levelled against the FDA by Mr Ralph Nader and his colleagues (see *Nature*, April 18, page 205) may be correct. And although Dr Edwards's colleagues and associates insist that he is doing a splendid job, it is plain that there is a long way to go before he can put his house in order.

The most serious bone of contention last week was the way in which arrangements have been made to allow soft drinks and other commodities containing cyclamates to be reintroduced to the market in the United States by classifying them not as foods but as non-prescription drugs. The Fountain committee and the FDA are united in believing that cyclamates are a hazard to health, but Dr Edwards and his staff seem to have been at once inconsistent and rather less than their own masters in devising the arrangements by which many products containing cyclamates will be kept on the shelves of many supermarkets when the supposed ban on their sale becomes effective by the simple device of relabelling them as drugs. Mr Fountain said that the episode "gives the impression of being a subterfuge to keep cyclamates in circulation", and that his only comfort was the FDA's promise to look at the position within a year to make sure that the availability of cyclamates would not be abused.

The day-long row about cyclamates was evidently regarded by the committee not merely as an issue in its own right but as a test of Dr Edwards's intentions to make the FDA into a vigorous and independent organization, and everybody agrees that he has a Herculean task on his hands. Indeed, the inquiry opened with Dr Edwards's own declaration that when he was appointed to the FDA on December 15 last year, the scientific competence of the FDA was "poorly marshalled", with a "clear imbalance between scientific and regulatory responsibilities. The decision making environment was disorderly. Professional talent was stifled, poorly directed and unmotivated. Planning was shockingly myopic".

In the reform of the FDA, Dr Edwards said that he plans to increase the degree to which it relies on consultation from outside, and he said that the National Academy of Sciences–National Research Council has already agreed to do even more work than at present on the revision of the list of food additives permitted for use (the GRAS list) and on its continuing study of drug efficacy. To review the balance of the FDA's internal operations, an independent scientific committee has been established, while permanent advisory councils will be set up to advise the three bureaux (Food, Drugs and Pesticides). Evidently in an attempt to anticipate the sudden crises which have afflicted his predecessors, Dr Edwards has set up what he calls a "critical reporting procedure" by means of which professional people within the organization can bring potential difficulties to his attention.

Ralph Nader.

The independence of the FDA was one of the preoccupations of the subcommittee. Who is the boss, the Commissioner of the FDA or the Secretary of Health, Education and Welfare ? One of the inevitable difficulties is that the FDA differs from most regulatory agencies in being responsible to a cabinet officer and not to Congress. Dr Edwards said last week that he would not have taken his new job if he had not been assured of having sole responsibility for administering the law on food, drugs and cosmetics, but he was quickly tripped up by a quotation from a letter on the FDA files in which one member of the staff, anticipating problems likely to arise during a meeting on cyclamates with industrial representatives, suggested that "it is important that industry should do most of the talking" and that the officials of the FDA should insist that they were merely advisers to the Secretary of HEW. The truth, it seems, is that Dr Edwards agreed before he joined that cyclamates were a special case and that higher authorities would make the important decisions. Dr Edwards also volunteered that he has an agreement with the Secretary's office, chiefly in the persons of Dr J. Steinfield, the Surgeon General, and Dr Roger O. Egeberg, Assistant Secretary for Health and Scientific Affairs, to provide advance warning of FDA decisions likely to cause trouble, but he also argued that the right of access to the Secretary which had been established gave his agency enough strength to be able to "move in the direction" of what one committee member called maximum freedom.

Within the agency, Dr Edwards said that his policy was to "push down" the level of decision making, and that formal studies were now being made of the procedures which would be necessary to allow decentralization of procedures such as the rejection of applications from manufacturers for approval for food additives and drugs. Dr Edwards was frank about the extent to which staffing problems hamper this process, and said that "because of poor management within the agency, people have been badly used". Dr Edwards also agreed with a suggestion by one of the committee that public service regulations make it hard to deploy people in a way that is likely to make the best use of them—"This has been a problem in the past five months. It does slow things down. The regulations do make people too secure. The contrast with private industry is striking."

But may not the FDA be hamstrung by too little money ? Dr Edwards was eloquent on the subject—"we are one of the badly funded agencies in the federal government and you can't regulate an industry with a turnover of more than $20,000 million on a budget of between $80 and $90 million a year". In reply to several questions, Dr Edwards thought that the discussions now under way with the Bureau of the Budget would mean that the FDA would have to drop some of its plans for communicating more effectively with the medical profession, but he did promise that priority would be given to the revision of the GRAS list on which work has already begun and the strengthening of the FDA's work in carcinogenesis and teratogenic chemicals.

The plea for more money did not instantly melt the heart of the subcommittee's chairman, who asked that the FDA should pay more attention to the enforcement of the law, if necessary at the expense of its scientific work. He pointed out that between 1945 and 1968, the FDA budget had grown from $4 million to $66 million and its payroll from 850 to 5,100 people. In the same interval, however, the number of occasions on which FDA inspectors had seized consignments of food had declined from 2,504 to 384 and drugs failing to satisfy the regulations had been seized on 594 occasions in 1945 but on only ten in 1968. Mr Fountain held that these statistics were not so much a proof of the FDA's success—one interpretation of them—but of a turning away from the strictly regulatory aspects of its work. "The reason for the FDA's decline may be attributed in part to the morale of FDA personnel", itself a product of frequent changes of leadership, frequent reorganization, physical moves from one building to another, programmes begun and not completed, fear of displacement because of frequent changes and uncertainty as to FDA enforcement programmes and policies. Dr Edwards agreed that "the morale of the agency has been low and is still low, but we're dedicated to making it a topflight agency".

Much of the discussion at the inquiry last week concerned the way in which the FDA has set about trying to make sure that all purchasers of contraceptive pills are warned of the potential danger. This has been a running sore ever since last March, when the FDA's proposal for a statement of the dangers running to 600 words found its way into several newspapers in the United States. According to Dr Edwards, the intention then was to make sure not merely that women using the pill were aware that these materials are potentially hazardous but also to provide them with statements of the possible side effects, contraindications to the use of contraceptive pills, a list of signs of possible trouble, advice about the use of contraceptive pills in several circumstances, as for example during lactation, and a description of the long term experiments being carried out to test for the occurrence of cancer in laboratory animals. By Dr Edwards's account, the appearance of this long statement led to such a degree of protest from medical bodies and pharmaceutical manufacturers that he became convinced that it was not for the FDA to play doctor. The result, announced last week, is that in future (or after the 90 days during which interested parties may object) all packets of oral contraceptives will contain a simple statement advising women not to take the drug without continued medical supervision and advising them that a fuller statement, prepared by the manufacturer, will be available from physicians. He promised that the FDA would be vigilant in making sure that the new booklets were an adequate primer in the theory and practice of oral contraception.

Here again, so the inquiry last week suggested, the FDA's policy tends to be fuzzy at the edges. One question, for example, is whether it is possible to assume that all women using oral contraceptives, either now or in the future, will have access to medical supervision or, for that matter, whether they will be able to read English. At one point, Dr Edwards found himself admitting that large numbers of women obtained supplies of oral contraceptives by sharing a common prescription, although he considered that out and out illegal traffic in oral contraceptives is probably negligible. But why, then, back down from the policy that "the public has a right to know about the benefits and the side effects", and why not consult the medical advisory committee on the form of words now to be inserted in packages of oral contraceptives ? Plainly the FDA has bowed to the wind blowing from outside as well as within the Department of Health, Education and Welfare.

In the past few months, the FDA has also been plagued by criticisms of having suppressed or even perverted the results of scientific studies carried out in its own laboratories. On this point, both the FDA and the subcommittee agreed that there is no substantial case to answer. The chief source of these accusations is Dr Howard Richardson, a pathologist at

FDA, who made public earlier this year charges that some of the early studies on the potential damage done by cyclamates may have been falsified. According to Dr Edwards, there has since been a thorough study of Dr Richardson's charges, from which it has emerged that one of the cases in which information is alleged to have been suppressed consisted of a study in 1950 in which cyclamates were fed to a strain of rats with a congenitally high incidence of lymphosarcoma. According to Dr Edwards, both the experimental rats and the control group emerged with the same incidence of tumours, for which reason the pathologists concerned did not consider the result significant. Mr Fountain announced that his own staff, following up the same allegation, had discovered that the pathologists whose scientific studies were alleged by Dr Richardson to have been falsified or suppressed had since dissociated themselves from the charges.

But where does the FDA stand on the Delaney amendment ? How vigorously, in other words, is it prepared to enforce that piece of the law which holds that any substance fed to any experimental animal by mouth and which is afterwards found to cause cancer should be prohibited from use in human foodstuffs ? This has been an important question in the past few weeks, if only because the Department of Health, Education and Welfare has let it be known that it is impatient with the rigours of the Delaney amendment, the grounds on which it was necessary last year to ban cyclamates (see *Nature*, **224**, 398; 1969). Dr Edwards said last week, "I think there are some improvements to be made, but until I have had more time for study" the ban must stay. Of the discussions being held within the Department of Health, Education and Welfare about the Delaney amendment, he said that he believed both Dr Steinfield and Dr Egeberg were anxious to "strengthen" the Delaney amendment and not to weaken it, by which he meant, if a prolonged and uncomfortable period of questioning is to be taken at its face value, that the Department of Health, Education and Welfare is about to propose that mutagenicity and teratogenicity will be added to the present requirements that foodstuffs should lack carcinogenicity.

The saga of the cyclamates occupied the whole of the committee's time one day last week. On the whole, the FDA was able convincingly to demonstrate that there was no reason for removing cyclamates from the GRAS list until the late sixties. Admitting that the GRAS list should include only substances about which there was no reasonable doubt, however, Dr Edwards found himself listening to a long recitation from the chairman of occasions dating back to October 30, 1967, on which apparently responsible bodies, including the Food Additives Committee of the National Research Council, had raised questions about the safety of cyclamates. Dr Virgil Wodicka, director of the Bureau of Foods, Pesticides and Product Safety, pointed out that the interpretation of preliminary results is necessarily difficult and Mr William Goodrich, Assistant General Counsel to the FDA, pointed out that much of the early research was supported by the Sugar Foundation and that "their lawyers bombarded me with all kinds of arguments". According to Mr Goodrich, the proposal to remove cyclamates from the GRAS list first arose in April 1969. Dr Edwards considered that it should have been possible to prohibit their general use before October 15, 1969, although he "wouldn't say quite when".

The tale of precisely how the ban was announced, recited by Mr Goodrich, sounded last week a sufficient explanation of the confusion which followed. The results of the feeding experiments carried out by the Abbott Laboratories in which mixtures of cyclamate and saccharin were fed to rats were said to have reached the FDA on a Monday morning and were considered both by the Surgeon General and by officials of the National Cancer Institute. A meeting of an advisory committee arranged for the Thursday of that week was provided not merely with a presentation by the Abbott Laboratories but also with an analysis of the microscopic slides of supposed bladder cancer in the experimental rats. In the course of 36 hours, the advisory committee was apparently convinced that cyclamates should be removed from the GRAS list and the decision to do so was approved by the Secretary of the Department of Health, Education and Welfare, Mr Robert Finch, on the Friday afternoon and was made public at a press conference the following day.

What has followed since seems to be the blackest mark in the FDA's new record. According to evidence at the inquiry last week, principally that of Mr Goodrich, an *ad hoc* committee called by Dr Egeberg recommended cyclamates should be made available as non-prescription drugs for use by diabetics and obese people. (Insulin is one of the non-prescription drugs on sale in the United States.) Mr Goodrich said that he was "called upon" to see whether this decision could be implemented legally and that he decided there would be no objection to making materials containing cyclamates available. The question with which the committee eventually defeated the FDA was whether it is possible to exempt a substance from the provisions of the law about food safety simply by labelling it a drug—one of the documents in the case is one of the FDA's own regulations which lays down that an application for the approval of a new drug (among which the cyclamates are now numbered) must also be considered as an application for the approval of a food additive if the substance concerned materially changes the characteristics of the food. But there are also doubts about the fairness of marketing foodstuffs including cyclamates in circumstances in which they will be placed on supermarket shelves more or less in their old familiar places and freely available to all purchasers. Given the way in which it has also been arranged that applications for the new licences to market cyclamates will be processed not by the FDA in Washington but by the regional offices, which will have power merely to approve the cautionary label to appear on containers, it is no wonder that Mr Fountain complained that it was hard to understand the rationale of the FDA's procedures. The explanation is probably that provided by Dr John Jennings, Associate Commissioner for Medical Affairs, who explained that applications for registering cyclamate products as new drugs "did not require scientific data about efficacy or safety, that question having been resolved for better or worse". Evidently this was an occasion on which the lawyers and not the scientists made policy. Dr Edwards's success in the months ahead will no doubt depend on the extent to which he can assert the right of technical people to make technical judgments.

PERUVIAN EARTHQUAKE

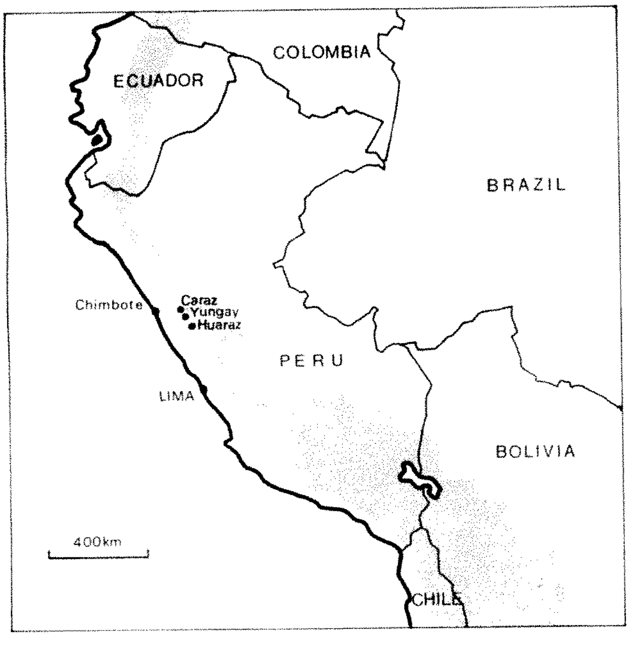
Price of New Sea Floor

from our Geophysics Correspondent

THE earthquake in Peru in June may well have claimed more than 50,000 victims and the number could be much greater. The earthquake is thus comparable with the most catastrophic known—the Lisbon earthquake of 1775, which claimed about 50,000 lives, and the Kwanto earthquake of 1923, which killed 100,000. The epicentral region was about 300 miles north of Lima and the damage wrecked four cities, one, Chimbote, on the coast and three, Caraz, Yungay and Huaraz, about 75 miles inland. Several other villages in the same canyon as these three cities were also wrecked. Two mountain ranges, the Negra and Blanco ranges, rise up on each side of the canyon. The access roads have been destroyed and reaching the region by air has been made impossible by the flooding of the only airport in the region at Caraz. It seems likely that much of the damage was done by the rupturing of the walls of a dammed lake, Lake Llanganuco.

The magnitude of the event was recorded as 7·75 on the Richter scale, a figure which can only be an approximation because such a huge disturbance drives seismometers off scale practically all over the world. The figure is somewhat smaller than that for the San Francisco earthquake of 1906 and that in Alaska of 1964, but the damage, of course, is determined much more closely by the relative positions of epicentre and the centres of population. Thus an earthquake of comparable magnitude in the Aleutian islands in 1965 drew no attention at all because of its remoteness.

The cause of the circum-Pacific earthquakes is now fairly well understood. New sea floor is created along the East Pacific Rise, a mid-ocean ridge running from south of Australia to Mexico. More, in fact, than just sea floor appears to be created—the outer 50 to 100 km of the Earth, called the lithosphere or plate, is formed by solidification of molten regions along the rise and lateral transport away from the region of creation of new plate. The sources of energy for this process, while almost certainly convective, have not yielded to any simple approach to the problem. The East Pacific plate (in this case) moves eastwards at about 5 cm/yr until it encounters the leading edge of western South America, which is part of the South Atlantic plate. Just offshore, the plate is bent through 45° (the source of much seismic activity), underrides the Andes (this is probably the most destructive region) and continues to show sporadic activity down to several hundred kilometres along this dipping plane. The deep ocean trench just off the South American coast is undoubtedly a surface manifestation of the start of the downward plunge.



The sliding of one plate past another is a potentially dangerous process. The San Andreas fault in California is an example of such sliding along a vertical fault plane. The sliding occurs along the 45° dipping plane under South America and in many other regions round the Pacific. It seems that in many regions this relative motion of a few centimetres a year is accommodated by some sort of lubrication (ground water has been suggested) or by repeated small earthquakes, but certain regions (well known in California but practically unmapped elsewhere) stick for periods of tens of years while the stresses build up. Strain energy can be stored in the vicinity of these locked faults until the relative displacements between two points far from the fault reaches ten metres, but then stresses exceed the elastic limit and a catastrophic breakdown occurs along the fault plane with the surrounding region jumping to its new unstrained position. A large number of smaller aftershocks usually occur as readjustment continues, and in Peru at least 100 aftershocks have been reported. It is likely that the width of the region of accumulated stress in this case was about 100 km parallel to the coast line and the length of the fault down the dip was between 20 and 50 km. The Chile earthquake of 1960, somewhat larger, appeared to have a width of about 1,000 km.

Does a huge earthquake in Peru cause other earthquakes, or does it release strain and reduce the chances of an earthquake elsewhere? As far as anyone can tell, the answer to both these questions is no. There has been some speculation that one earthquake may trigger another, but the evidence is slight beyond the region of local strain readjustment (perhaps 1,000 km).

Could such an event have been predicted or even controlled? This is a topic which is receiving growing attention in the United States where the problem in California is more severe than many are prepared to acknowledge. Research around the San Andreas fault is beginning to pinpoint the regions of large strain accumulation where the size of the future earthquake grows with every year that it is postponed. But although it is known that some regions have large amounts of potential energy stored in them, premonitory signs are very difficult to come by. There is some evidence of dramatic strain changes before earthquakes and, rather surprisingly, of very small magnetic fluctuations, but the science is a long way from being sufficiently well established to deliver credible warnings. On the control side, some very interesting developments are occurring with fluid injection experiments. These are in an early stage but seem to bear promise that regular lubrication of a fault would encourage more frequent small slippages. All this, of course, is cold comfort for the residents of South America and Japan where the faults are rarely accessible and there is certainly no money for even pilot experiments in fault lubrication.

NEWS AND VIEWS

Antigens Unmasked

EVEN the most imperceptive observer must have realized that research into the structure, composition and function of cell membranes is very much in vogue. Numerous molecular biologists, bored with *E. coli* and its viruses, can be found toying with red blood cells, retina, synapses and the like, all of them materials which only two or three years ago were strictly left to the physiologists. At the same time tumour virologists are beginning to pay increasing attention to the changes in the cell surface that accompany the transformation of cells by oncogenic viruses. It is now clear that transformation by the small DNA viruses SV40 and polyoma, the genomes of which specify probably less than ten proteins, results in surprisingly complex changes in the surface antigens of the transformed cells.

One of the more interesting ideas that has emerged to account for the complexity of these changes is that SV40 and polyoma viruses somehow induce the specific uncovering of antigenic components which are present in the membranes of untransformed cells but are masked and therefore not accessible to the immune system. Häyry and Defendi (*Virology*, **41**, 22; 1970), for example, report the detection, by mixed haemagglutination and indirect immunofluorescence, of a specific surface (S) antigen on SV40 transformed cells. This antigen is present neither on the untransformed cells nor on cells of the same lines transformed by polyoma virus. To that extent the S antigen is specific to SV40 transformation, but it is not apparently a product of an SV40 gene. Mild short term digestion of untransformed or polyoma transformed cells with trypsin or chymotrypsin, but not papain, ficin or neuraminidase, exposes the S antigen characteristic of SV40 transformation.

The S antigen does not seem to be related to other changes in the cell surface which accompany transformation. It does not, for example, cross react with the Forsmann antigen, the amount of which is known to increase after transformation with polyoma or SV40 viruses. It is not apparently related to the sites which bind wheat germ lipase agglutinin and it is not hematoside, which is present in a masked form on the surfaces of untransformed cells and in an incomplete form on transformed cells.

Ben-Bassat, Inbar and Sachs (*Virology*, **40**, 854; 1970) have followed the appearance of another change in the surface of cells transformed with SV40. Last year Sachs's group reported that 85 per cent of the sites capable of binding concanavalin A are cryptic on untransformed cells but become exposed after transformation. They now claim that cells must divide at least once after SV40 infection and reach a density of 10^5 cells per cm^2 before this exposure of cryptic sites occurs. After infection with SV40 many of the cells which bind concanavalin A are abortively rather than stably transformed. As these abortively transformed cells continue to divide they lose the transformed cell phenotype and the binding sites revert to the cryptic state. If, however, they are maintained in conditions which inhibit cell division they retain the capacity to bind concanavalin. The exposure of these binding sites is a phenotypic characteristic of all transformation by SV40 but a genotypic character only of stably transformed cells. Obviously, just how a small oncogenic viral genome brings about such striking changes in the surface of its host will be one of the chief preoccupations of tumour virologists in the next few years.

PROTEINS

Assorted Assembly

from our Molecular Biology Correspondent

SELF-ASSEMBLY is still probably one of the most potent totems to let slip in one's grant applications, and T4 phage is a favoured example. The phage head is a system of considerable complexity, and the interdependence of its several protein components has been demonstrated by the study of mutants with a malfunction in one or other gene. The latest chapter in this long story, unfolded over the years largely by Kellenberger and his associates, is to be found in an article by Yanagida *et al.* (*J. Mol. Biol.*, **50**, 35; 1970). Seven genes have been implicated in defining the phage head structure, and the function, or at least the consequences of malfunction, of five of them has been determined. One gene, designated 23, is responsible for the predominant subunit protein, whereas others determine the elongation and shape of the head, and one seems to prevent a non-specific aggregation of the 23-protein. Among other deviant forms that have been recognized, an error in gene 22 causes the formation of long tubes, or "polyheads".

Yanagida *et al.* have studied the structure of the tubes by optical diffraction of electron microscope images. Earlier discrepancies are resolved by the demonstration that the tubes consist for the most part of several concentric layers, but that these tend to separate on lysing the cells, so that electron micrographs give the impression of particles of variable diameter. In the intact structures one sees always an inner shell of some 290 Å inside diameter. Successive layers number up to seven or more in some particles, and their separation is about 70 Å. The tubes bear a helical lattice pattern of variable pitch and a right-handed screw-sense.

Evidence has now appeared, however, of some unsuspected depths in the self-assembly process. For example, Larcom, Bendit and Mumma (*Virology*, **41**, 1; 1970) have been exercised to establish the molecular weights of the proteins. Molecular weight determinations on a polydisperse mixture of unfractionated proteins (in a non-ideal system to boot) is not usually a very rewarding occupation, but Larcom *et al.* estimate that in a dissociation, denaturing solvent, guanidine hydrochloride, a mixture of large and small components is present, the first having a reported molecular weight of 78,000. A partial chromatographic separation by size was also performed. The central finding in this work is that when a reducing agent, mercaptoethanol, is added to the denaturing solvent, the molecular weight of the large component drops to an estimated 48,000. This also happens when the sulphydryl groups in the protein are first chemically blocked, and it is suggested that disulphide-linked dimers may be present in the phage head.

These conclusions seem at odds with the results of work in other laboratories, and the molecular weight of the large component, which must comprise chiefly the 23-protein, has to be seen against a remarkable report by Kellenberger and Kellenberger–van der Kamp (*FEBS Lett.*, **8**, 140; 1970). They estimate molecular weights by the empirical but so far remarkably successful method of electrophoresis in polyacrylamide gels containing the detergent, sodium dodecyl sulphate. When the gene (number 31) for the "solubilizing protein" is rendered inactive by mutation, lumpy aggregates of the 23-protein appear in the head envelopes. When the protein is extracted from these, or from the tubular polyheads (gene 20 silent), a molecular weight of 61,000 is derived. Normal capsids, however, lead to a value for the same component of 49,000. The difference persists after treatment with guanidine hydrochloride and mercaptoethanol. Although it is still far from proven, the most convincing explanation is that one of the head proteins, functioning as a protease, causes the loss of a fragment of the 23-chain during the final stage of assembly. Components corresponding in size to the molecular weight deficiency have in fact been observed in the gel electrophoresis. If this scheme turns out to be correct, it will make an interesting dent in the mystique of self-assembly, which in this case at least could no longer be seen as a thermodynamically reversible process, governed only by the intrinsic physical properties of the proteins in the system.

PHOTOBIOLOGY

Co-factors and Chloroplasts

from a Correspondent

THE biochemistry of electron transport was the principal theme of the meeting of the British Photobiology Society at King's College, London, on June 10. Dr A. Trebst (Ruhr University, Bochum) described the isolation of a chloroplast component (S_L-eth) which seems to function in the electron transport chain between photosystem 1 and ferredoxin. The component was isolated using two antibodies to chloroplasts which inhibit ferredoxin-dependent reaction; one apparently reacts with the complete co-factor, the other with its prosthetic group. The co-factor, which, contrary to earlier reports, seems to be identical to San Pietro's ferredoxin reducing substance, has a molecular weight of 5,000. It is spectroscopically undistinguished, with a single absorption peak in the 260–270 nm region. The nature of the prosthetic group is not yet known.

Dr D. A. Walker (Imperial College, London) and Dr J. Wiskich (University of Bristol) described experiments on oxygen evolution by "whole" chloroplasts. Walker proposed that a criterion for the "wholeness" of chloroplasts in a suspension might be their ability to use flavin-adenine dinucleotide (FAD) as electron acceptor, intact chloroplasts being unable to take up FAD. He presented evidence that intact chloroplasts are also impermeable to adenosine triphosphate (ATP) and nicotinamide–adenine dinucleotide phosphate (NADP). Wiskich presented data on control ratio and P/2e ratio (1·6) in unbroken pea chloroplasts. These chloroplasts would not meet Walker's criteria for intactness, and the dangers of measuring P/2e ratio and other parameters in mixed populations of whole and broken chloroplasts were discussed.

Drs M. C. W. Evans and R. V. Smith (King's College, London) described the preparation of cell free nitrogenase from the photosynthetic bacterium *Chloropseudomonas ethylicum* and the blue–green alga *Anabaena cylindrica*, and the relationship of nitrogenase to the photochemical system. Nitrogenase from both organisms is similar to that from non-photosynthetic bacteria requiring ATP and a low potential reductant. In *Cps. ethylicum* the reductant may be ferredoxin, photoreduced by photosynthetically active particles from the bacterium. Reduced ferredoxin does not function with nitrogenase from *Anabaena* and the nature of the natural electron donor is unclear.

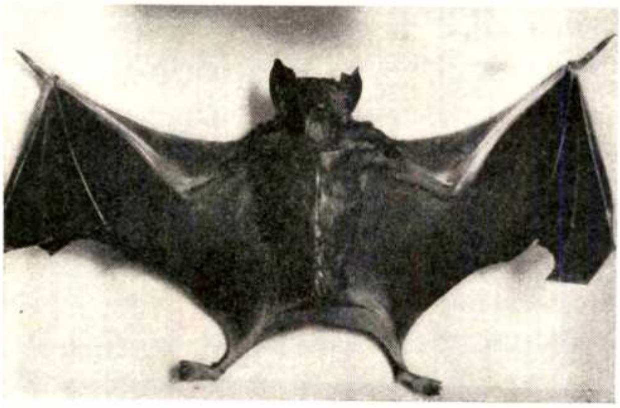
Dr A. R. Crofts (University of Bristol) reported the investigation of rapid *p*H changes induced in chromatophores of *Rhodopseudomonas spheroides* by illumination with laser and xenon arc flash. Using mutants deficient in carotenoids it was possible to correlate the rapid binding of protons, measured using bromocresol purple as indicator, with electron transport in the chromatophores measured by changes in the redox state of cytochrome *b*. It was found that 1 proton was bound per 120 chlorophyll molecules in the untreated chromatophores or 2 in the presence of valinomycin and antimycin A. The results were interpreted as indicating the transport of protons in accord with the chemiosmotic hypothesis involving two chromatophore components.

ZOOLOGY

Research at Regent's Park

VAMPIRE bats stole the limelight when the Zoological Society of London held the first open day at its animal hospital and research institutes in Regent's Park on June 11. Although in Trinidad the blood sucking vampire bats are pests, constantly pursued by the government bat catchers, the colony at the Nuffield Institute of Comparative Medicine is valued for the properties of its saliva. A wound made by a vampire bat continues to bleed long after the beast has bitten because of the action of the saliva. Dr T. Cartwright and his colleagues have found that the saliva contains an enzyme, named desmokinase because of the bat's other name, *Desmodus rotundus*, which activates plasminogen to plasmin, which in turn breaks down blood clots.

Desmokinase, with a molecular weight of about 2 million, seems to be a proteolytic enzyme which cleaves a protecting peptide from the active site of plasminogen. The attack of desmokinase on blood clots *in vitro* proved more effective than that of the other activators of plasminogen now in use—urokinase and streptokinase. But purification of desmokinase is still in progress and there will be a long time to wait before clinical trials can begin on a potential new treatment for sufferers from thrombosis.



Vampire bat.

Schoolboys and students, too, can expect to benefit from the fruits of the labours in the Nuffield Institute, for Dr G. H. du Boulay is preparing an atlas of radiograms of the cerebral arteries of mammals. So far he has injected the arteries of dead specimens of more than a hundred species and made radiograms from them. Early volumes are in the press, and when the atlas is complete its author hopes that it will become a revered guide for dissection, updating the classic work of nineteenth century anatomists.

Activity in the Wellcome Institute of Comparative Physiology centres on reproduction in various exotic animals which have not previously been familiar inhabitants of laboratories. Dr B. J. Weir has a collection of hystricomorph rodents from South America, including the tuco-tuco (*Ctenomys talarum*) which unexpectedly developed diabetes in captivity. The degu (*Octodon degus*) bred so successfully that its numbers increased seven-fold in its first year of captivity. The chinchilla (*Chinchilla langer*), however, is far less fertile, which leads to problems for commercial breeders. The reproductive difficulties of chinchillas arise because they have long heat cycles within a restricted breeding cycle, and also have a long pregnancy, so that opportunities for conception are limited. Pregnant mare's serum and human chorionic gonadotrophin have been injected to induce fertility, with the consequence that successful mating was followed by normal pregnancy and the birth of normal numbers of young.

The next step is to inseminate semen artificially from chosen males to propagate desired characteristics of fur quality and reproductive capacity. Advice will doubtless be on hand from Dr R. C. Jones, who is studying the problems of preserving mammalian semen for artificial insemination. Although many millions of cows have been inseminated with deep frozen sperm, the semen of some species deteriorates unless it is used when fresh. Dr Jones is looking at the injurious effects of freezing on sperm of various animals, including zebra, chimpanzee and boar, a species which presents particular problems of sperm preservation. Artificial insemination may well provide the means of preserving species in danger of extinction.

POLLUTION

Take a Deep Breath

from a Correspondent

CONCERN for the effects of atmospheric pollutants on the lung was reflected in the theme of the annual Hanford Biology Symposium, held from June 2 to 5 at Richland, Washington. A notable feature of the meeting was the increasing interest in attempts to define effects of pollutants not only in terms of pulmonary structural and functional changes affecting gas exchange, but also in terms of the underlying biochemistry. The cells in the lung have always seemed to biochemists much less active and interesting than cells of the liver, kidney, heart, brain and other organs, but now lung biochemistry has emerged as a discipline in its own right. Several contributions were devoted to analyses of metabolic pathways in lung cells, which have certain peculiarities.

Much attention was given to lipid synthesis in the lung in an attempt to understand the formation and turnover of the surfactant which, by reducing surface tension at the liquid–air interface, helps to maintain open airways. One of the pioneers of this work, Dr J. A. Clements (University of California, San Francisco), reported that in the lungs of eleven vertebrates, varying from reptiles to man, the abundance of surfactant and its principal component, dipalmitoylphosphatidylcholine, is related to the area of the respiratory surface, whereas the quantities of other lipids reflect pulmonary mass and diet. How many surfactant components exist is still unknown, nor has its composition provided clues to differences in susceptibility to adverse environments—although surfactants represent an obvious possible target. Several investigators, notably Dr J. Gil (University of Berne, Switzerland), have developed methods for preserving a layer of material over the alveolar epithelium in electron micrographs, and most experts agreed that this probably corresponds to surfactant. Dr O. K. Reiss (University of Colorado) and his colleagues have shown that a specific thiolacetate esterase is liberated from the lamellar bodies of the type 2 epithelial cells and is apparently related to surfactant.

Several contributors dealt with the origin and biochemistry of alveolar macrophages. Dr M. A. Brunstetter and her colleagues (University of California, Davis) used chimaeric mice with esterase (*Es*-2) markers to show that most alveolar macrophages originate from bone marrow. Several pollutants have been found to affect the capacity of alveolar macrophages to kill organisms, this being the principal mechanism by which the lung resists bacterial infections. Some of the most striking effects on macrophages are produced by particulate pollutants, such as silica and asbestos, as described by Dr A. C. Allison (Clinical Research Centre, London).

Of the gaseous pollutants, most work has so far been carried out with oxidizing atmospheres, such as high oxygen, ozone or nitrogen dioxide. These oxidize lipids and produce marked changes in lung structure and function. The structural changes following exposure to pure oxygen at one atmosphere, described by Dr E. R. Weibel (University of Berne, Switzerland), included degeneration of endothelial and type I alveolar epithelial cells and replacement of the latter by type II epithelial cells.

GALAXY FORMATION

Link with Expansion of Universe

from our Cosmology Correspondent

THE plausibility of the Eddington–Lemaître equations as representing the true mathematical description of our universe has been considerably enhanced by calculations which show that not only can galaxies form and grow in such a universe in a reasonable time, but that this process is itself inextricably linked with the expansion of the universe. Since Lifshitz's work more than twenty years ago, galaxy formation has posed a considerable problem to cosmologists, because it seemed clear that the universe just is not old enough for galaxies to have evolved from local condensations in an approximately homogeneous cloud of gas. This has caused models to be suggested in which either more complex cosmologies are required or special conditions are imposed to enable galaxies to grow. Recently R. Simon has developed the proposal of W. C. Saslaw that long range forces of gravitational attraction may be important in the growth of irregularities, producing density waves with wavelength close to the Jeans length.

Simon's calculations confirm this possibility, but of even greater significance is his discovery that another peak in the spectrum of possible density waves becomes significant for late epochs in the history of the universe. Unlike the peak corresponding to the Jeans length, which remains constant and can produce condensations of around galactic mass, the second peak moves in the direction of larger wavelength as the universe evolves and becomes increasingly significant (*Astron. Astrophys.*, **6**, 151; 1970). It is tempting to interpret this behaviour as implying that the galaxies formed by the condensations due to the first wave will become clustered about the maxima in the second wave, and that these clusters of galaxies will move away from each other (with relative velocities proportional to their separations) due to the increase in wavelength of the second wave. Clearly this picture is most agreeably consistent with observations, while providing the long sought after clue as to why the Eddington–Lemaître model affords a good description of the universe.

Detailed questions of why galaxies have the variety of structure which is observed, and how stars form from the protogalaxies, remain to puzzle theorists interested in the formation of galaxies.

COMETS

Silicate Grains in Comet Bennett

by our Astronomy Correspondent

THE infrared astronomy group at the University of Minnesota, last year one of the teams that presented evidence for the presence of silicate grains in the interstellar dust (*Nature*, **222**, 326; 1969), has reported what seems to be the silicate emission feature in the spectrum of Comet Bennett (R. W. Maas, E. P. Ney and N. J. Woolf, *Astrophys. J. Lett.*, **160**, L101; 1970). The comet was observed on April 4 with a thirty inch telescope at wavelengths between 2 and 20 microns, and an emission feature was detected at 10 microns. This seems to be the feature already detected in emission from cool stars and from the Orion nebula, and in absorption in the spectrum of the cool supergiant 119 Tauri. In a series of papers in *Astrophys. J. Lett.* last year the feature at 10 microns was attributed to the presence of silicate grains (**155**, 181–199; 1969).

The experiment on Comet Bennett is interpreted by the Minnesota group as confirmation that the 10 micron feature is indeed probably due to a silicate or a combination of silicates. The association between comets and meteor streams—which has long been well established—indicates the probable presence of silicate grains within comets. Although the finely divided particles which make up meteor streams are almost certainly in a different class from the more substantial objects which survive the flight through the atmosphere and which have a known composition, evidence for silicate elements in shower meteors comes from the optical spectra seen as meteors burn up in the atmosphere. Thus the detection of the 10 micron feature in a location where silicates are expected is accepted by the Minnesota group as adding some weight to the silicate hypothesis. But in line with the current view that interstellar dust is as likely as not a mixture, chiefly of silicates and graphite, the group say their infrared data point to a second refractory material in the nucleus of Comet Bennett which could easily be carbon.

HUBBLE SEQUENCE

New Light on Galaxy Evolution

from our Cosmology Correspondent

THE evolution of galaxies and the reasons for the existence of the different categories of galaxies have yet to be explained with anything like the confidence which can be placed in the accepted models of stellar evolution. But the progress being made in the investigation of these problems is emphasized by P. Brosche, who has incorporated several recent developments in a model where the Hubble sequence is interpreted as an angular momentum sequence of galaxies of roughly equal mass (*Astron. Astrophys.*, **6**, 240; 1970).

It is assumed that star formation simply occurs when the gas making up a protogalaxy becomes sufficiently dense for local regions to collapse under their self gravitation, and on this interpretation protogalaxies with small angular momenta shrink relatively rapidly, becoming relatively dense and converting most of their mass into stars before they can become highly flattened, so that they form the elliptical nebulae. The galaxies with more angular momentum convert only a small percentage of their mass into stars before they become highly flattened, resulting in a distribution of stars within a disk as seen in the spiral nebulae. Models of this type require a mechanism for the release of energy during the collapse of a protogalaxy, and in this particular case the efficiency of the mechanism must be linked to the angular momentum of the protogalaxy. Brosche favours a process involving collisions of interstellar gas clouds, the number densities of which have been estimated as 10^6 per kiloparsec for our galaxy. The energy loss considered is the macroscopic kinetic energy of these gas clouds, not the thermal energy of the molecules, so that quasi-static evolution rather than a violent collapse is certainly plausible. Although many aspects of Brosche's work are so far rather unsophisticated, it seems to be pointing in a highly profitable direction. Measurements of the rotational velocities of elliptical galaxies (if not quasars!) and more advanced *N*-body calculations are the obvious means whereby both observational astronomers and theoreticians can contribute to its further development.

COMA CLUSTER

Missing Mass still a Problem

from our Observatories Correspondent

G. and A. de Vaucouleurs of the McDonald Observatory (University of Texas) have reported the results of a search for luminous matter between galaxies in the Coma cluster (*Astrophys. Lett.*, **3**, 219; 1970). Coma is one of the best examples of a cluster in which there is a "missing mass" problem. Measurements of the redshifts and projected separations of member galaxies have been used to infer the mass of the cluster through the virial theorem. This assumes, of course, that the cluster is a stable, self-gravitating system. The mass obtained from the virial theorem is about 7×10^{14} Suns, while the total mass of the visible galaxies is estimated to be about a factor of five less than this. Discrepancies of this kind are commonly found in clusters of galaxies. But whereas most clusters of galaxies appear as loose aggregates with little central condensation, the Coma cluster is a radially symmetrical, centrally condensed object which certainly looks to be in equilibrium.

There are several possible forms for the missing mass: intergalactic stars, neutral hydrogen, ionized hydrogen, molecular hydrogen and "black holes" have all been suggested. Some of these have already been ruled out. For example, neutral hydrogen in sufficient quantity would have been detected by its 21 cm emission, and ionized hydrogen hotter than a million degrees would produce detectable X-rays.

The de Vaucouleurs searched for luminous matter—emitting gas or intergalactic stars—by scanning across a diameter of the cluster, which is about a degree across, with a sensitive pulse-counting photometer. They determined the mean sky background well away from the cluster and found that in blue light this was equivalent to one star of magnitude 22·3 every square second of arc. This background is mostly due to terrestrial airglow rather than to the integrated luminosity of faint stars and galaxies. A small excess luminosity extending over 30–45 minutes of arc centred near the geometrical centre of the cluster was detected. The peak of this excess brightness was only 26·2 magnitudes/s^2. The overall "intergalactic" luminosity amounted to 40 per cent of the accepted value for the integrated luminosity of cluster galaxies, however.

This excess luminosity is not interpreted as being truly intergalactic (due to stars which are not bound to any particular galaxy). Instead, the de Vaucouleurs conclude that it can be substantially accounted for by the overlapping coronas of the two supergiant elliptical galaxies, NGC 4874 and NGC 4889, which lie at the centre of the cluster, and a few others. Measuring the total luminosity of a galaxy is a long standing problem in astronomy. As Hubble found, the luminosity per unit area goes down almost as the square of the radius in the outer parts of a galaxy, so that it merges imperceptibly into the background sky with a still substantial contribution coming from the outer parts. The de Vaucouleurs estimate the effect of the corona of NGC 4889 by comparing the luminosity profile of its inner parts with that of the nearby, isolated, supergiant elliptical, M 87. The luminosity profiles agree when suitable allowance is made for the different distances of the two galaxies. Recent work by Arp and Bertola, and by G. de Vaucouleurs, on M 87 itself showed that this galaxy, which on photographs has an apparent diameter of less than 4 minutes of arc, is in fact at least a degree across. Others had used sky comparison areas about 30 minutes of arc away from M 87 and the revised measurements raised its total luminosity by half a magnitude. The de Vaucouleurs' measurements of the Coma cluster led them to increase the luminosities of the two supergiant galaxies in the Coma cluster by the same factor.

The upward revision by 40 per cent of the total light from galaxies in the Coma cluster does little to relieve the "missing mass" problem. The mass-to-light ratio required to bind the cluster is still about 200 solar units. For normal elliptical galaxies with mass and light distributions that have been measured, this number does not exceed about 50.

ISLAND MOVEMENT

Dissent on Madagascar

from our Geomagnetism Correspondent

THE assertion by Strong and Flower (*Earth Planet. Sci. Lett.*, **7**, 47; 1969) that Madagascar has been in its present position relative to Africa since the late Carboniferous, and probably since the Pre-Cambrian, has been strongly criticized by Wright and McCurry (*Earth Planet. Sci. Lett.*, **8**, 2671; 1970). Strong and Flower based their conclusion on the existence of sandstone inclusions in some of the lava flows on the Comores Archipelago which separates the Mozambique Channel from the Somali Basin (see map, *Nature*, **225**, 11; 1970). This, they claimed, provides "unequivocal evidence that this part of the Indian Ocean is underlain by non-oceanic crust".

Wright and McCurry disagree with this. They suggest that slightly metamorphosed sandstone inclusions in basalts of the Comores Archipelago are evidence only that beneath some of the volcanoes there are arenaceous sediments, fragments of which have been carried to the surface and baked by the ascending lavas. In short, they feel Strong and Flower draw too strong a conclusion. They agree that the evidence against a southward movement of Madagascar, in particular the fact that the sense of displacement (sinistral) along fracture zones in this area of the Indian Ocean is in the wrong direction, is too strong to be denied—but disagree that the island has thus stayed put. They point out that a case may still be made for an older proposal that Madagascar followed India and the Seychelles north-eastwards, having originally occupied the Inhambane delta region north of Lourenço Marques. Although this interpretation conflicts with some of the geological evidence, it is consistent with the sense of the transcurrent faulting in the South-West Indian Ocean and yet is not inconsistent with the presence of sandstone inclusions in the Comores lavas. The debate will no doubt continue.

RNA at Cold Spring Harbor

from our Special Correspondent

THE view that classical molecular biology is played out, with no significant questions awaiting answer, was refuted by this year's symposium at Cold Spring Harbor on RNA transcription. With a wide range of systems covered, new control elements in bacteria and bacteriophages were reported as well as new enzyme activities in mammalian cells.

The sequences of the single stranded RNA phage R17 now known amount to almost 500 of 3,500 nucleotides (Fig. 1) and those of *E. coli* 16S ribosomal RNA to 200 out of 1,600 residues. The first hundred nucleotides from the 5′ end of R17 comprise a sequence which cannot code for any of the three proteins which the phage specifies, but S. Cory, J. M. Adams and P. Spahr (Institut de Biologie Moléculaire, Geneva) were not too surprised by this finding, for much the same is true for another RNA phage, Qβ. The last fifty nucleotides at the 3′ end of the R17 phage may also remain untranslated. The only termination sequence so far determined (for the coat protein gene) has two successive nonsense codons; the 3′ terminal region has no such sequence and so cannot specify protein if a double terminator is a general requirement.

Comparison of the 5′ terminal sequence of R17 with that of Qβ shows a surprising degree of homology—surprising because the two phages do not seem to be related serologically. The 5′ terminal sequence of R17 probably folds into two hairpin loops. The first (residues 1–29) shows extensive homology with the starting region of Qβ. The sequence of the second loop of R17 (residues 37–74) is also homologous with Qβ, not with the analogous region but with a sequence between Qβ residues 101 and 128. This suggests that R17 and Qβ may have descended from a common ancestor, a region of which was deleted in R17 at some point after evolutionary separation. The conservation of sequence at the immediate 5′ terminal region suggests that, although not translated into protein, this region must have some function which is so important for the phage that it has been preserved through evolution.

Another remarkable homology is that between the 3′ terminal sequences of the plus and minus strands of the phage. In phage replication, an RNA replicase enzyme attaches to the 3′ end of the infecting (+) strand and synthesizes a complementary (−) strand. When this is complete, the enzyme attaches to the minus 3′ end and synthesizes a plus strand. Only the plus strand has been sequenced directly, but the nucleotide sequence of the minus strand can be deduced from its complementarity with the plus strand.

Both strands have an identical sequence of six nucleotides at their 3′ terminus

$$\begin{array}{r|c|l} \cline{2-2} \ldots\ldots\ldots\ \mathrm{G\,U\,U\,A} & \mathrm{C\,C\,A\,C\,C\,C} & \mathrm{A{-}OH}\ \ 3'\ \text{plus} \\ \ldots\ldots\ldots\ \mathrm{G\,G\,U\,C} & \mathrm{C\,C\,A\,C\,C\,C} & \mathrm{C{-}OH}\ \ 3'\ \text{minus} \\ \cline{2-2} \end{array}$$

and this common sequence may be the recognition site for the RNA replicase. This is supported by the finding that, although the last 3′ nucleotide can be removed without affecting the infectivity of the phage, any further degradation causes a large decrease in infectivity. The proposed sequence is just large enough to provide sufficient specificity; any given six nucleotide sequence would occur about one in four thousand times by chance, and the phage is some 3,500 nucleotides long.

Sequences of more of the coat protein gene were reported by P. G. N. Jeppesen, J. L. Nichols, F. Sanger and B. G. Barrell (MRC Laboratory of Molecular Biology, Cambridge). It now looks as though much of the phage forms hairpin loops. The RNA folds back on itself in at least three parts of the internal regions of this gene and also at its beginning and end. It seems likely that this reflects a need to pack the RNA within its protein coat, and there is speculation that it may also be used as a mechanism for controlling its translation.

16S ribosomal RNA from *E. coli* contains not only hairpins but also the looped regions joined by double stranded stems characteristic of transfer RNA. P. Fellner, C. Ehresmann and J. P. Ebel (Laboratoire de Chimie Biologique, Strasbourg) reported that the 200 residues so far sequenced suggest that the rRNA may have a structure much like a giant tRNA.

Sigma Action

The symposium showed that the control of transcription is turning out to be a complicated process which may involve any of the subunits of RNA polymerase. The *E. coli* enzyme consists of four types of subunits associated in the structure $\alpha_2\beta\beta'\sigma$. Sigma determines the specificity of initiation in bacterial cells and is released from the DNA-enzyme complex shortly after initiation, leaving the $\alpha_2\beta\beta'$ core enzyme to elongate the RNA chain. How does sigma control the initiation of transcription? When core enzyme transcribes DNA *in vitro*, it initiates RNA synthesis at random sites. When sigma is added, initiation is restricted to certain sites—probably those which are used *in vivo*. D. Hinckle and M. Chamberlin (University of California, Berkeley) reported that core enzyme by itself

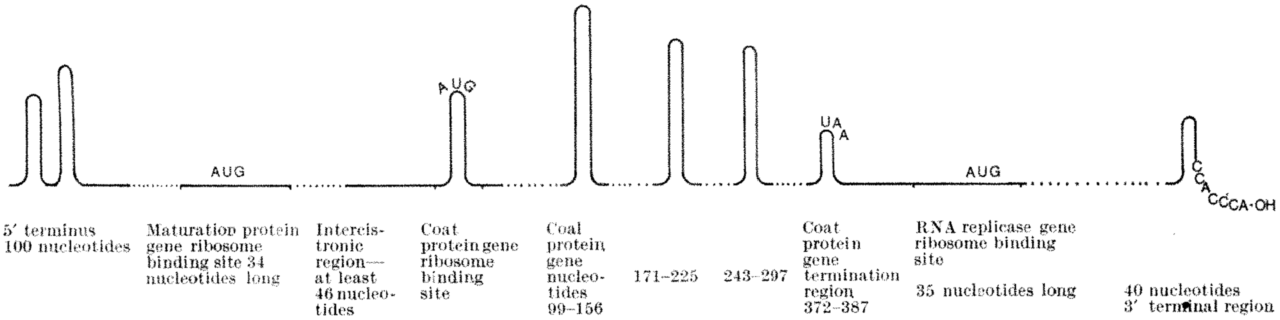

AUG
AUG
UA A
AUG
CCACCCA·OH
5′ terminus 100 nucleotides
Maturation protein gene ribosome binding site 34 nucleotides long
Intercis-tronic region—at least 46 nucleotides
Coat protein gene ribosome binding site
Coat protein gene nucleotides 99–156
171–225
243–297
Coat protein gene termination region 372–387
RNA replicase gene ribosome binding site
35 nucleotides long
40 nucleotides 3′ terminal region

Fig. 1. Secondary structure of RNA phage R17 for the 500 nucleotides which have been sequenced. The phage is about 3,500 nucleotides long and the drawing is not to scale. About 70 per cent of its length probably forms base paired loops.

associates non-specifically with DNA in a loose binding which is rapidly reversible. Sometimes, by chance, the core will bind at a promotor site (where synthesis is initiated *in vivo*). Interaction with sigma forms a more stable complex in which the enzyme is tightly bound and can no longer dissociate from the DNA. This ensures preferential initiation of transcription from tight binding sites.

It is not clear exactly how sigma stabilizes the complex. Hinckle and Chamberlin suggested that it may either itself directly recognize the nucleotide base-pair sequence of the promotor site, or do so indirectly by effecting an allosteric change in the core when this is located at a promotor. But W. Zillig *et al.* (Max Planck Institut für Biochemie) showed that instead it recognizes an initiation site which is adjacent to the primary binding site. Both groups, however, agreed that sigma opens the DNA duplex for the start of transcription. Initiation depends on temperature in a way which suggests that the first step in RNA synthesis is the action of sigma to melt the DNA to produce a region of local unwinding.

Although attempts to discover more than one kind of sigma factor in *E. coli* have failed, the production of new sigma-like factors plays an important part in controlling the development of bacteriophages. Immediately after entry into a bacterium, phage T4 DNA is transcribed by the host RNA polymerase. This transcription is specific for the "immediate early" genes of the phage. If chloramphenicol is added, these genes are still transcribed, but a second class of early genes, the "delayed early", can no longer synthesize RNA. This suggests that their transcription requires the synthesis of a phage-coded protein, and this has proved to be a sigma-like factor which directs the core enzyme of the host to transcribe the delayed early and also some of the immediate early genes.

Subunits of the core enzyme are also changed during infection. The α subunit is modified early on, but this does not affect the specificity of transcription. The β′ subunit is modified later, about 15 min after infection. A. A. Travers (Harvard University) reported that, after this modification, the core has a much lower affinity for sigma, and this is probably responsible for the shut-off of early genes which occurs at this time. Late genes can be transcribed only after a second phage-coded sigma-like factor has been synthesized; this directs the modified core to synthesize late mRNA from T4 DNA *in vitro*.

But *in vivo*, transcription of late genes seems to be coupled to DNA replication. E. P. Geidescheck *et al.* (University of Chicago) have found that when temperature sensitive mutants in the gene which codes for the phage DNA polymerase are raised to the non-permissive temperature, late mRNA synthesis is reduced by 90 per cent as soon as replication ceases. The transcription depends on concurrent replication, and the phage DNA goes through a cycle in which it enters a competent state when it is replicated and soon becomes incompetent again. This suggests that it is physical state of the DNA which is important. The use of ligase mutants showed that the action of this enzyme is needed to convert the DNA to the incompetent state. This suggests that transcription of late genes requires the gaps in the DNA which are produced by discontinuous replication. One model to reconcile the *in vitro* and *in vivo* results suggests that the T4-early sigma-like factor cannot be displaced from the core *in vivo* by the late sigma-like factor. Binding to gaps in the DNA may effect release of the early sigma, thus permitting its replacement by the late sigma.

Sporulation in *B. subtilis* also demands changes both in a sigma-like initiating factor and in core subunits. R. Losick, A. L. Sonnenshein and R. L. Shorenstein (Harvard University) have used the bacteriophage φe which grows on vegetative but not on sporulating bacteria. *In vitro*, RNA polymerase extracted from vegetative cells, but not from sporulating cells, can transcribe the phage. Although the vegetative core enzyme does not transcribe φe DNA, activity is restored by addition of either vegetative *B. subtilis* initiating factor or *E. coli* sigma factor. When enzyme from sporulating bacteria was used, neither initiating factor nor sigma factor enabled the sporulation core to synthesize RNA. Comparison of the subunits of the two cores showed that one of the B subunits of the vegetative enzyme was replaced in the sporulation enzyme by a different polypeptide with a molecular weight reduced from 155,000 to 110,000. This change probably accounts for the switching off of vegetative genes at sporulation. Losick is now looking for a new initiating factor which will use the modified core to turn the sporulation genes on.

Termination of Transcription

Hopes that the action of the rho factor might provide a simple model to explain all terminations of RNA synthesis were dashed at the symposium. To be sure, rho interacts with RNA polymerase at specific sites on the DNA template to release RNA chains *in vitro*, but specific termination can also take place *in vitro* in the absence of rho.

Transcription of lambda DNA *in vitro* is initiated at two sites, one on the *l* strand and one on the *r* strand. J. W. Roberts (MRC Laboratory of Molecular Biology, Cambridge) reported evidence from Harvard that, in the absence of rho factor, both RNA products remain associated with the fast-sedimenting transcription complex. When rho is added, transcription is terminated at a specific site on each strand to release the distinct RNA species characterized as 12S and 7S.

Rho has much the same effect with phage T4 DNA as template *in vitro*, if the salt concentration is low. J. P. Richardson (University of Washington) found that rho limits transcription to the immediate early genes. When the termination factor is omitted from the reaction mixture, the RNA products are larger and contain sequences in addition to those representing the immediate early genes; this is probably caused by the RNA polymerase reading past the termination signals.

Increasing the concentration of KCl, however, decreased the efficiency of rho-mediated termination, and above 0·15 M KCl, the same products were made with or without rho. But termination can still be specific even in the absence of rho. U. Maitra, A. Guha and A. Lockwood (Albert Einstein College of Medicine) showed that termination on T4 DNA *in vitro* takes place at specific sites when the salt concentration is high, but not when it is low. Rho is not needed for this effect.

After the complexity of the larger bacteriophages, reports of work with phage T7 introduced a note of refreshing simplicity. T7 DNA has a molecular weight of 26×10^6 and complementation mapping shows that there are at least nineteen genes. The last 0·7 per cent of the phage is terminally redundant, but is not circularly permuted (Fig. 2). Transcription is totally asymmetric and takes place only from the *r* strand. W. C. Summers and R. B. Siegel (Yale University) have found that there are two stages of RNA synthesis during infection. The shift from early to late synthesis requires the product of gene 1; this is probably a sigma factor which redirects

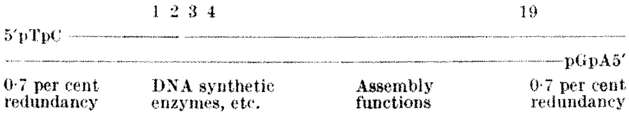


Fig. 2. Bacteriophage T7.

the activity of host core enzyme, for *in vitro*, *E. coli* RNA polymerase transcribes only early genes and the enzyme from T7-infected cells transcribes late genes. Sufficient mRNA is synthesized by transcription of only the infecting parental strand, with no need for replication of the phage; this RNA is remarkably stable for a bacterial system, with a half life of more than 15 min.

Initiation of synthesis of early RNA takes place at only one site on T7 DNA, yet Summers and Siegel have found four early RNA species in infected cells, of molecular weights 1·1, 0·45, 0·29, $0·20 \times 10^6$. A very different result is obtained *in vitro*. R. L. Millette and C. D. Trotter (University of Colorado) reported that, in a system without rho, T7 DNA directs the synthesis of only one RNA species, which has a molecular weight of about $2·1 \times 10^6$. This roughly totals the molecular weights of the four early RNAs.

Two types of termination may therefore be taking place *in vivo*. As RNA polymerase transcribes the DNA from the single initiation point, rho terminates transcription at each of three successive sites, but the enzyme does not dissociate from the DNA template. This termination might be achieved either by rho breaking the RNA chain at specified points after its transcription, or by a genuine termination event. The latter would require the RNA polymerase to re-initiate synthesis at an adjacent site, but without the benefit of sigma factor. When the polymerase reaches the fourth termination site, transcription is terminated and the enzyme dissociates from its template in reaction which does not need rho. The combination of hybridization techniques with electron microscopy provided another approach to this system. R. W. Davis and R. W. Hyman (California Institute of Technology) presented electron micrographs which showed the sites where particular RNA species hybridize to the DNA. When T7 DNA is visualized after denaturation and random renaturation, a tangle is seen. But if RNA is hybridized to the DNA, the DNA–RNA hybrid regions cannot renature, although the remaining regions of single stranded DNA can do so. The sites of hybridization can be located by using suitable visual markers on the DNA.

Native T7 DNA early after infection shows a clustering of RNA polymerase molecules at one end of the genome. This single initiation site is located about 1·3 per cent from the end of the DNA, so that it is outside the region of terminal redundancy. Early RNA hybridizes only with this end of the phage DNA. The number of polymerases attached to the DNA increases at the rate of about one a minute for about 15 min, and synthesis of RNA takes place at about forty-five nucleotides per second. The whole template can be transcribed into one large messenger; this is complete after about 25 min.

There is no termination of transcription in this system. This can be reconciled with the discrete messengers produced in other systems, however, because these use different strains of phage T7. Although all T7 strains derive from a single plaque (set up in 1947), there has been divergence into at least six strains. The course of infection is much the same irrespective of strain, but Davis and Hyman's strain seems to lack the termination–dissociation signal of Summers and Siegel's and Millette and Trotter's strain.

Looking at RNA Synthesis

Seeing is believing was also the theme of the most spectacular contribution to the symposium, from O. L. Miller and B. R. Beatty (Oak Ridge National Laboratory) and B. A. Hamalko and C. A. Thomas (Harvard University). Their visualizations of nucleolar DNA transcribing ribosomal RNA have been extended to bacterial systems. After rupture by osmotic shocks, the intracellular contents of *E. coli* fragile mutants were examined by electron microscopy.

Fig. 3 shows a thread of DNA running horizontally, with RNA strands attached to it at intervals. Each RNA chain meets the DNA template at a granule which is about the right size to be an RNA polymerase enzyme. All the RNA chains under transcription have ribosomes attached to them in fairly close packing; because virtually no free polysomes were seen, it is likely that the translation of messengers can take place only while they are themselves under synthesis. An operon can be defined by this technique as the region between the shortest polyribosome (the promotor end) and the longest (the terminator end).

How do these results correlate with earlier biochemical studies? Most operons seen in these pictures have about ten messengers attached at random intervals, with the largest polyribosome containing up to about twenty ribosomes. Just before the presentation of this paper, J. K. Rose, R. D. Mosteller, R. A. Baker and C. Yanofsky (Stanford University) announced a re-appraisal of some of the parameters of the *E. coli* tryptophan system. Their previous estimates that about three messengers each bearing ninety ribosomes should be attached to the operon at any give time have now been revised to suggest about fifteen messengers with up to twenty ribosomes each. The definite periodicity of transcription hitherto observed has proved unreproducible, and can now only be observed occasionally. This revision gives excellent agreement between the results obtained by biochemical and visual techniques.

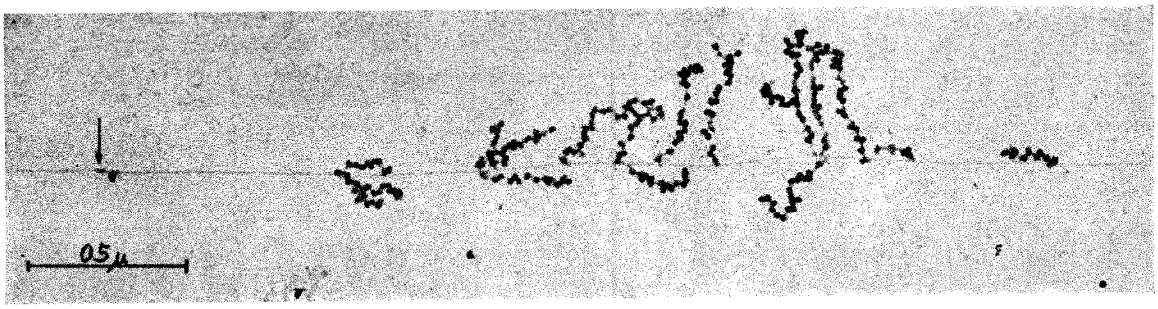


Fig. 3. Visualization of an operon of *E. coli*.

Control for Lactose Operon

Induction of the *lac* operon of *E. coli* requires 3′–5′ cyclic AMP both *in vitro* and *in vivo*. Among defective mutants which cannot respond to the nucleotide, R. L. Perlman *et al.* (National Institutes of Health) and G. Zubay (Columbia University) have identified strains which lack a protein which binds cyclic AMP. This protein, which may be partially purified from wild type bacteria, restores the ability of *in vitro* extracts of mutant cultures to induce the operon. It seems to act on RNA synthesis, consistent with its stimulation *in vivo* of transcription from the promotor.

It is difficult to pin down exactly what is happening in these systems because both transcription and translation are taking place. L. Eron reported that Beckwith's group at Harvard University have established a system which looks only at transcription. RNA is transcribed from a preparation of DNA of phage φ80 carrying the lactose genes. It is then hybridized with DNA from a lambda phage which bears the lactose genes. Because there is virtually no homology between the φ80 and lambda phages themselves, only RNA transcribed from the lactose genes can anneal to the lambda strands.

Transcription in this system depends completely on the presence of sigma factor. But even with the complete RNA polymerase, transcription is symmetrical and both DNA strands direct RNA synthesis. Addition of cyclic AMP and the partially purified binding protein, however, restricts transcription of the lactose operon to the correct strand of DNA. Nevertheless, it is not yet clear whether the system is working as it does *in vivo*. There is some suggestion that transcription may be initiated not at the *lac* promotor but at a site to the left of the regulator *i* gene. Another cause for disquiet is that transcription is not repressed by addition of the *lac* repressor. All the same, it is now clear that control of the lactose operon is more complicated than thought hitherto, and must require at least one more protein control factor.

Multiple RNA Polymerases

One session of the symposium was devoted to what was to prove essentially a workshop on mammalian RNA polymerases. Eleven groups reported their success in purifying the true polymerases. Although there were some reports that there may be three or even more distinct polymerases in animal cells, there was general agreement that there are two nuclear polymerases. One is associated specifically with the nucleolus and transcribes ribosomal RNA and the other is in the nucleoplasm and, presumably, makes messenger RNA.

Many papers concerned with bacterial RNA polymerase referred to the "wonder-drug" rifampicin which binds to the β′ subunit and inhibits transcription at or soon after initiation. Because chain elongation is not affected, the continued transcription of RNA in the presence of rifampicin implies that it must have been initiated before the drug was added. Unfortunately, its effect is confined to the bacterial enzyme; a counterpart for mammalian systems, however, was reported, and although α-amanitin has not yet earned the title of wonder-drug, it is being widely studied.

Its mode of action is not known, if only because the concentrations at which it inhibits different systems vary over at least a ten-fold range. But there is agreement that the type I enzyme, from the nucleolus, is not inhibited, while the type II enzyme, from the nucleoplasm, cannot synthesize RNA after addition of α-amanitin. Use of this criterion shows that comparable enzymes are present in all the various animal cell types studied.

Another cytological observation made its contribution to molecular biology in the discussion on mammalian polymerases. Addition of α-amanitin to salivary glands prevents the formation of puffs along the chromosome (presumably where messenger RNA is synthesized), but does not inhibit the synthesis of RNA at the nucleolus (which continues to incorporate a precursor into RNA).

Central Dogma Inverted

Reports of new types of enzyme activities filled the closing sessions of the symposium. A. J. Shatkin and A. K. Bannerjee (Roche Institute of Molecular Biology), and W. K. Joklik, J. J. Shehel and H. J. Zweerink (Duke University Medical Center, North Carolina) reported that the virion of reovirus (a double stranded RNA virus) contains an RNA-dependent RNA polymerase which synthesizes RNA by a completely conservative mechanism; it is analogous to DNA-dependent RNA polymerase and transcribes messenger from the genome without affecting the integrity of the template. Presumably, another enzyme must be responsible for replicating the RNA parental duplex by a semi-conservative mechanism.

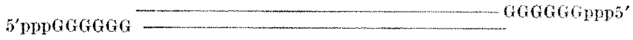


Fig. 4. Segments of the genome of reovirus.

Reovirus is also notable for the state of its genome, which was shown by S. Millward (Wistar Institute, Philadelphia) to comprise ten different segments which can be separated on polyacrylamide gels. Each segment has a 5′ end which consists of a single stranded length of about six purines, probably guanines (Fig. 4). This means that the different segments cannot join together, either covalently or by overlapping of complementary single-stranded sticky ends. How the virus selects one copy of each segment for its virion is now under study.

The discovery that RNA can act as template to direct the synthesis of DNA provided a dramatic finish for the symposium. It has been known for some time that inhibitors of DNA synthesis can prevent the successful infection of animal cells by certain RNA viruses. D. Baltimore (Massachusetts Institute of Technology) reported results similar to those discussed by Temin at Houston (*Nature*, **226**, 1003; 1970). Baltimore found that an RNA-dependent DNA polymerase activity is located inside the virion of Rausch mouse leukaemia virus and Rous sarcoma virus (both single stranded RNA viruses). The enzyme is presumably coded by the virus.

What sort of DNA does this enzyme synthesize ? It seems likely that the immediate product is a DNA–RNA hybrid. This might then synthesize a duplex DNA by displacement of the RNA strand, and such DNA could replicate and might be integrated into the genome of the host cell. It could also direct RNA synthesis, as indeed might the DNA–RNA hybrid itself.

Earlier, H. Stein and P. Hausen (Max Planck Institut für Biologie) had reported that, when purifying a factor that inhibited RNA synthesis on denatured DNA templates, they found an enzyme which degrades DNA–RNA hybrids. Its action is a specific degradation of the RNA strand only. The apparent absence of any function *in vivo* for this enzyme may have been filled by Baltimore's study; perhaps it is concerned in some way with defending animal cells against viral invaders.

Solar Eclipse of March 7, 1970

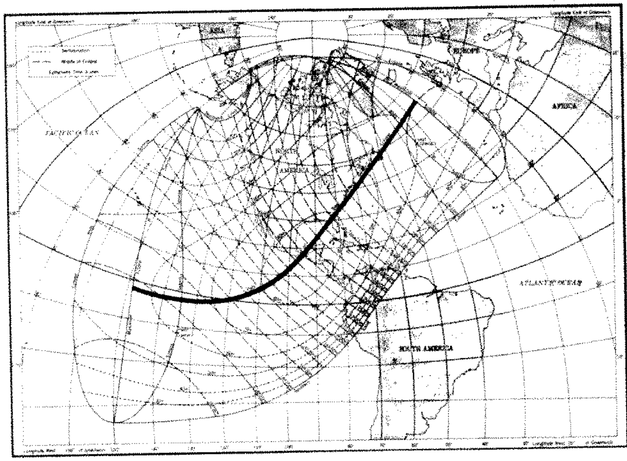
Eclipse not Spoilt by Shortage of Funds

The total eclipse of the Sun on March 7 this year was obviously a great adventure for everybody who took part. For many people the search for an observing site where the probability of clear skies would be greatest meant a trip to central America, where the path of the eclipse crossed southern Mexico before sweeping over the Gulf of Mexico and north along the east coast of the United States, Nova Scotia and Newfoundland, coming to an unremarked end in the North Atlantic halfway to Ireland. But the most bizarre anecdote belongs to the Naval Research Laboratory. They had to call in the salvage department of the United States navy when their rocket experiment to catch the ultraviolet flash spectrum of the Sun sank in the Atlantic after a parachute failure. Fifteen days after the eclipse the payload was rescued from a mile beneath the sea, with one set of photographs miraculously still intact (see page 1132).

Why do people still go to such lengths to record an eclipse ? After all, it must count as one of the longest standing activities of the astronomer. A hundred years ago in the columns of *Nature*, Norman Lockyer was canvassing support for expeditions to Spain and to Sicily to observe the eclipse of December 1870, when the burning question apart from whether government help would be forthcoming was the nature of the corona. Surely by now solar eclipses must have been squeezed dry of all the information that they have to give ?

The reports in the pages that follow show how far this is from being the case. For a start, the perfection of the rocket has revitalized the study of the Sun as it has the rest of astronomy. By recording the spectrum of the rim of the Sun as the Moon inches its way to second contact, the properties of successively higher layers in the chromosphere can be determined. With guidance systems now available so that a rocket can latch on to the decreasing crescent of the eclipsed Sun the flash spectrum can be recorded into the ultraviolet. This was the impetus behind the Naval Research Laboratory experiment, and behind a more successful experiment involving an international group from Britain, Canada and the United States (see *Nature*, **226**, 249; 1970).

Since the 1920s, and the work of Appleton in particular, eclipses of the Sun have come to be red letter days for atmospheric scientists as well as astronomers. Indeed, nearly half of the reports which follow are to do with what happens to the atmosphere, chiefly the ionized part, when the solar radiation is blotted out. Partly as a result of soundings during solar eclipses it is now well established that the lower ionized layers, the D-region at around 75 km altitude and the E-region around 110 km, are maintained by solar X-rays and ultraviolet, and that the F layers which are at their most prominent near 170 km (the F1-region) and near 300 km (the F2-region) are maintained by extremely short ultraviolet radiation. Obviously the eclipsing Moon is a first rate piece of apparatus by which the solar radiation is rapidly obliterated so that the processes which govern the decay of the ionization can be studied without the complications appertaining at sunset. This time it was possible to measure how the total number of electrons in a column of the atmosphere was altered, by observing the Faraday effect on polarized signals from several geostationary satellites (see page 1111, for example). It will also no doubt turn out to be important that the Canadian satellites which use a miniature ionosonde to investigate the topmost reaches of the ionosphere—out of bounds for ionosondes on the ground because of absorption—were able to probe several areas in the penumbra. It is also splendid that the path of totality was sufficiently close to Puerto Rico to make it worth using the powerful radar at Arecibo to determine electron concentrations, and electron and ion temperatures throughout the atmosphere (see page 1124). This technique is based on the Thomson scattering of radar pulses by free electrons to obtain data over a wide spread of heights, but it requires very high power radar transmitters and large collecting surfaces such as the aerial at Arecibo, still sufficiently rare for people to be pleased that the penumbra crossed the Caribbean.



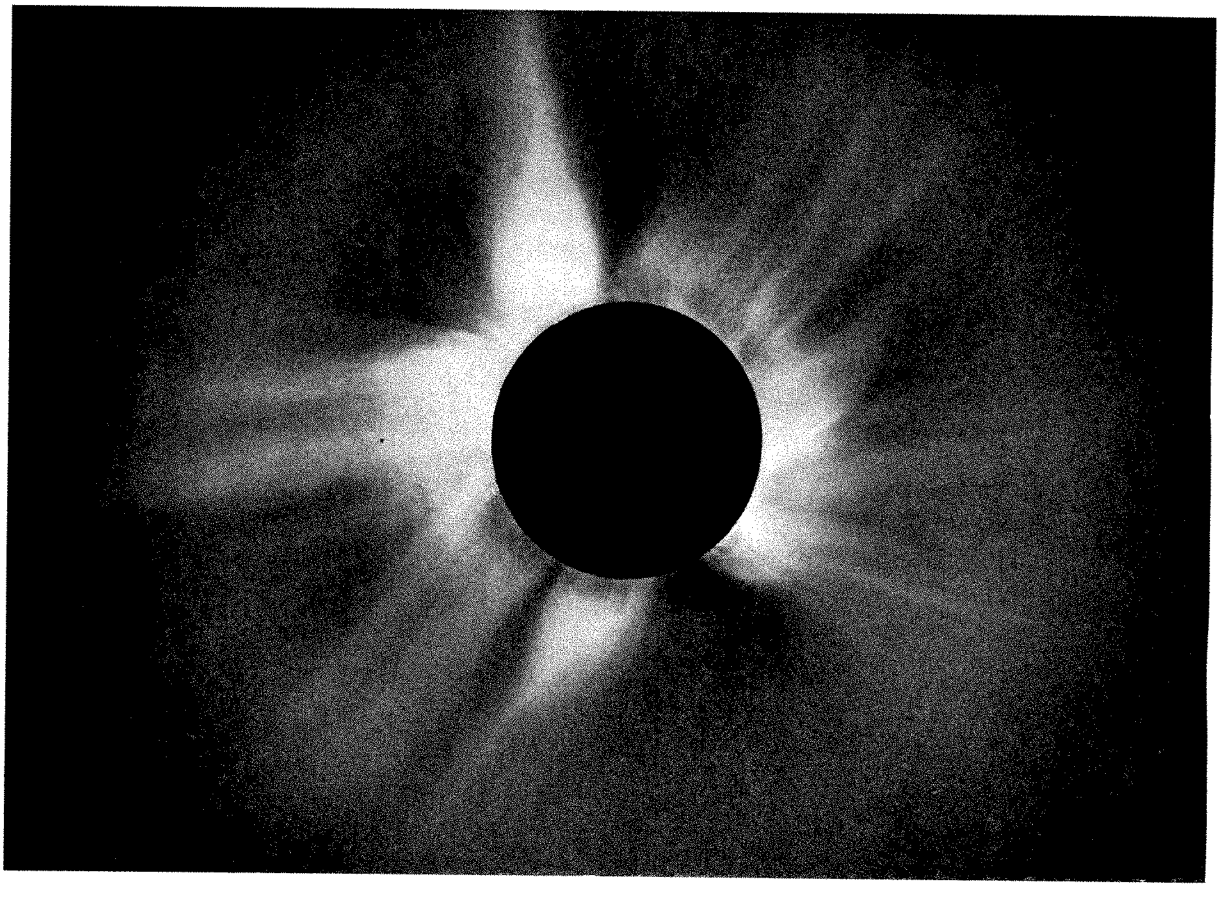
The path of totality, from *The American Ephemeris and Nautical Almanac.*

With experiments of this kind throwing light on the mechanisms governing the production and loss of ionization, and on transport processes in the ionosphere, it is satisfying that the March eclipse should have something to say about the way oscillations can be initiated in the atmosphere. Since 1960 when Hines resurrected interest in what are called atmospheric gravity waves—adiabatic waves for which gravity is the restoring force—the fashion has been to interpret the random motions which undeniably occur in the upper atmosphere in these terms, and it now seems that the perturbation of the atmosphere by the eclipse may have been enough to set up an oscillation (see page 1123). Indeed, the oscillation in the total number of electrons as recorded at Stanford uncannily matches the prediction of Chimonas and Hines that the curvature of the path of totality should tend to focus the waves on California.

The observers photographing the eclipse from the ground appeared to have benefited from unusually favourable conditions, which to begin with should lead to a better understanding of the solar corona. But the eclipse was not a carnival for astronomers only. On the eclipse day a research vessel from the Texas A & M University was out in the Gulf of Mexico to see how plankton reacted to the eclipse, if at all (see page 1155).

In an account of the North American eclipse of August 7, 1869, in the first issue of *Nature*, Lockyer said that the line of totality was almost one continuous observatory from the Pacific to the Atlantic, and something like that happened again in March. On this basis it can already be said that the observations on March 7 were a success, despite the parsimony that prevented the experiments from being as ambitious as might have been anticipated. One might have expected more advantage to be taken of the enormous good fortune of the rocket facilities at Wallops Island being close to the path of totality, for example, and it is a pity that there was insufficient money to make a supersonic observatory out of the aircraft that was to have chased the Moon's shadow and given more than an hour's worth of continuous totality.

The Corona during the March 7, 1970, Eclipse



THE photograph shows the solar corona observed about thirty seconds after second contact at the "High Point" camp (16° 20′ N, 6 h 25·0 m, altitude 8,800 feet) about thirty miles south-west of San Carlos, Yautepec, Mexico, by the eclipse expedition of the High Altitude Observatory of the National Center for Atmospheric Research*. The exposure was through a radially symmetric, neutral density filter in the focal plane to compensate for the steep decline of coronal radiance with increasing distance. This allows structural features to be traced from the chromosphere out to 4·5 solar radii on the original negative.

The long dimension of the frame is east–west (geocentric) and the bright helmet streamer with a prominence at its base is in the north-east (geocentric) at a heliocentric position angle of about 50°. Other bright helmet streamers appear at heliocentric positions 98°, 159°, 194° and 250°. The bright "horn" at 292° on the west limb appears on the original negatives to be due to the superposition on the line-of-sight of two fainter streamers. The corona at this time was unusual for the presence of streamers at high latitudes and the development of straight rays over the poles. The circumstances at the time of this eclipse were unusual for the rather dark sky. Long exposure photographs, made without the radially graded filter, show the lunar maria and suggest that the sky radiance did not exceed $3 \times 10^{-10} B_{\odot}$. Table 1 lists the technical data for the photograph.

Table 1. TECHNICAL DATA

Objective aperture	11·1 cm	Effective wavelength	6400 Å
Focal length	178 cm	Exposure	10 seconds
Focal ratio	*f*/15	Radial filter range	10^4 in transmission
Film	Kodak Linagraph Shellburst (70 mm)		

GORDON NEWKIRK, JUN.
LEON LACEY

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Eclipse Effects in the Ionosphere

by
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This article surveys some recent trends in the study of the ionospheric effects of solar eclipses.

UNTIL recent years the principal method of studying ionospheric eclipse phenomena was vertical incidence sounding by ionosondes, supplemented by recordings of fixed-frequency signals reflected from the ionosphere and measurements of radio-wave absorption. Early work is well documented in the record of a symposium held in 1955 (ref. 1); ref. 2 gives a more recent review. These methods are still important, but now three important new techniques are available. (*a*) Instrumented rockets can be launched at sufficiently short intervals to obtain several measurements of important parameters during the course of an eclipse. (*b*) The fortuitous passage of an artificial satellite through an eclipsed region can give useful data on the upper ionosphere. (*c*) Measurements of electron concentration, electron temperature and ion temperature can be made by the incoherent scatter technique: only a few installations exist, but by good fortune several observations of eclipses have been possible.

Modern computing methods have also made feasible the construction of good theoretical models which can be used to study the effects of various physical processes on the ionosphere.

The interpretation of eclipse phenomena in the ionosphere is often based on the continuity equation for the electron concentration $N(h,t)$, a function of height h and time t. The equation is

$$\partial N/\partial t = Eq - L(N) - \mathrm{div}(N\mathbf{V}) \qquad (1)$$

where q is the rate of production of ionization in the absence of the eclipse, and L is the rate of loss. The transport term $-\mathrm{div}(N\mathbf{V})$ expresses the effect of the drift velocity $\mathbf{V}$ of the ionization caused by diffusion, electric fields and neutral air winds. The "eclipse function" $E(h,t)$ is sometimes assumed equal to the geometrical fraction of the Sun's disk not obscured by the Moon, although as discussed later this assumption may not be justified. A particular reason for studying eclipses is that the production term Eq in equation (1) varies quite quickly in relation to ionospheric time constants, whereas the normal daily variation of q is slow (except near sunrise and sunset when the oblique incidence of the solar radiation makes detailed calculations of q very difficult).

Production and Loss in the Lower Ionosphere

In the lower ionosphere (D and E-regions) the transport term in equation (1) can usually be assumed to be unimportant, so that only information about production and loss is sought. In the D-region (below 90 km altitude) the chemistry is complicated, and the electron concentration is controlled, not only by photoionization and recombination, but also by various attachment and detachment processes that lead to the formation and destruction of negative ions. An eclipse affects both the photodetachment and photoionization rates, but not necessarily in the same way because of the different solar radiations involved. Thus a full understanding of D-region eclipse phenomena cannot be expected from measurements of electron concentration alone; other parameters—particularly fluxes of solar radiations—are needed also. Rocket observations provide a means for obtaining these data.

In the E-region, about 100–120 km, the chemistry is simpler, negative ions being scarce. Many analyses of eclipse data have been made with the aid of equation (1), but without the transport term. Usually a simple recombination law is assumed, $L(N) = \alpha N^2$; this is reasonable because the recombination coefficients α for the two chief E-region ions, NO^+ and O_2^+, are probably not very different. The lifetime of the ionization is only a few minutes, so the $N(t)$ variations respond rapidly to changes of production rate. Nevertheless the eclipse analyses have not led to accurate values of q and α. This is because, as X-ray pictures of the Sun clearly show[3], the sources of the X-ray emission responsible for most E-region ionization are unevenly distributed. Although ionospheric observations made at different sites during an eclipse have enabled localized sources to be located on the solar disk[4], nowadays more precise data are obtained by X-ray photography from rockets and satellites, which does not require an eclipse. Moreover, the interpretation of the ionospheric data is seriously affected by the residual production at totality, due to X-radiation from the uneclipsed corona[5]. Even if the eclipse function $E \simeq 0{\cdot}1$ at totality, as is probably the case, the derived values of α may be several times larger than would be obtained under the assumption $E = 0$.

Transport Processes in the F-region

In the F-region the lifetime of the ionization is comparable to the duration of the eclipse, and the production, loss and transport terms in equation (1) may all be of comparable importance. Above about 250 km the loss term takes the form $L(N) = \beta N$, with β decreasing upwards. It has been shown that the height-dependent loss coefficient can account for the appearance of an extra stratification, the so-called "eclipse F1½ layer", during some eclipses[5,6]. Recent calculations, however, suggest that drift motions also play an important part in the formation of the F1½ layer[7].

Eclipse effects in the F2 layer are complex, but recently they have been used to give information on transport processes, notably in the equatorial F2 layer which is strongly influenced by drift due to electric fields[8,9]. An observation of the drift motion was made during the November 1966 eclipse at Jicamarca, Peru, using the incoherent scatter technique (Peterson *et al.*, unpublished). The eclipse "punched a hole" in the lower F-region (200–250 km altitude) at about 0800 local time; the "hole" was observed to drift upwards at a speed of 50 m s^{-1}, reaching 600 km at about 1000 local time. This is roughly twice the upward drift speed observed on a normal morning at Jicamarca, as deduced from the Doppler shift of the incoherent scatter signal[10], so it is necessary to investigate whether the drift velocity would be influenced by the eclipse. This might happen if the eclipse disturbed the distribution of electrical conductivity in the E-region, where the electric field originates. It was indeed reported that E-region electric currents (detected by their magnetic effects at ground level) did change during this eclipse[11].

Skinner[12] has analysed Nigerian F2-layer data for the October 1959 eclipse by a method that takes account of vertical drift, obtaining values of q and β that are consistent with other information. The drift velocity is found to be 5–50 m s^{-1} downward at about 1200–1400 local time. This result disagrees with the observations of upward drift at Jicamarca just mentioned, and with the contemporary theory of the equatorial F2 layer, which requires upward drift by day. Clearly, further observations are required, and it would be interesting to apply Skinner's technique to extract drift velocities from a theoretical model in which a known upward drift is incorporated.

Sterling and Hanson[7] have made calculations with a theoretical model of the equatorial F2 layer, which incorporates upward drift as well as production, loss and diffusion. They analyse their theoretical eclipse $N(t)$ curves to recover values of q and β. If the drift is ignored in this analysis the resulting values of β are about three times too big, but if the drift is taken into account more correct values of β are obtained. In either case the analysis gives reasonably correct values of q.

F-region Temperature Changes

Increases of F2-layer electron concentration sometimes occur during eclipses. These have been attributed to thermal contraction resulting from the reduction of electron and ion temperature[13], although the temperature dependence of the loss coefficient β may be a contributory cause. Thermal contraction causes a downward displacement of the topside F2 layer, which has been observed by the Alouette I satellite[14].

The changes of F-region electron temperature are influenced by several factors, the relative importance of which may vary with latitude. An eclipse reduces the direct input of heat to the electron gas, but heat may continue to be supplied by photoelectrons produced outside the eclipsed zone and travelling along geomagnetic field lines. The rate of loss of heat to the ambient ions may change because it depends on the electron and ion concentration N. Loss of heat to the cooler lower atmosphere, by conduction in the electron gas, takes place only along magnetic field lines and therefore occurs most readily at high latitudes. Qualitatively these considerations may account for the fact that reductions of F-region electron temperature have been observed during eclipses at middle and high latitudes[13,15], whereas enhanced electron temperatures accompanied the reduction of electron concentration at Jicamarca in the November 1966 eclipse (Peterson, unpublished).

Some decrease of neutral air temperature in the F-region has been measured during an eclipse[16] and it may therefore be supposed that the eclipse will affect the neutral air winds, which also influence the F-region electron concentration. This is a topic requiring further investigation.

Summary

The chief objectives of ionospheric eclipse observations may thus be summarized as follows. (1) Investigation of the distribution of sources of ionizing radiation on the Sun, using the ionosphere as a "detector". This has now been superseded by direct ultraviolet and X-ray observations of the Sun. (2) Investigation of the production and loss processes in the ionosphere. Numerous complications arise concerning the lower ionosphere, but reasonably satisfactory results seem to be obtained from observations in the equatorial F2 layer, provided transport processes are taken into account. (3) Study of ionospheric transport processes in the ionosphere, particularly motions due to electric fields and temperature changes in the F-region, and associated changes in the neutral atmosphere. The recent developments mostly relate to these processes.

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Changes in the Lower Ionosphere during the Eclipse—A Preliminary Report of the Canadian Programme

Ground-based radio investigations and four rocket launches were carried out in Canada to study the effect of the eclipse on the solar radiation and electron densities in the lower ionosphere (below about 150 km). The following four articles describe the experiments.

(I) Introduction

A SOLAR eclipse provides an opportunity to study the effects on ionization densities in the lower ionosphere as the ionizing radiations are cut off. The effect should be to cause a return toward what is normally thought of as night-time conditions during the period of the eclipse. Differences in detail between the eclipse and the sunset change might be anticipated, however. During a normal sunset, atmospheric absorption progressively attenuates the various wavelengths responsible for electron production—first the short wavelength radiation is absorbed and lastly the long wavelength radiation—whereas all radiations are cut off simultaneously during an eclipse at a more or less constant solar zenith angle. If there are active regions on the Sun (sunspots), the covering and uncovering of the spots during the course of the eclipse provide an ideal situation for studying the effects of solar X-ray radiation on the ionization content of the D-region.

The effects of eclipses on the various ionospheric layers have been studied for many years[1]. Changes in ionization densities in the lower ionosphere are shown, somewhat indirectly, by observation of the phase and amplitude changes of long waves, and the observation of the change in attenuation of short waves reflected above the D-region. These results are difficult to interpret[2] and do not seem to have added much to our knowledge of the lower ionosphere. More recently, *in situ* observations by rocket-borne experiments have been reported for four eclipses: the eclipse of July 20, 1963, in Canada[3] (the electron densities for this eclipse were measured by a Langmuir probe and are therefore suspect at heights below about 90 km); the eclipse of May 30, 1965, in New Zealand[4] (this eclipse occurred near sunrise); the eclipse of February 12, 1966, in Brazil[5] and the eclipse of May 20, 1966, in Greece[6]. The *in situ* data of Kane[4,6] can be compared with the ground-based partial reflexion data of Gregory and Manson[7] and Haug *et al.* (private communication—the data are reproduced in ref. 8) obtained at nearby sites. A comparison of the time variation of electron density at 74 km during the May 20, 1966, solar eclipse in Greece, measured by the ground-based partial reflexion method and by the rocket-borne radio wave propagation method, shows reasonable agreement between the data, and a marked decrease by approximately a factor of seven, roughly centred on the visible eclipse. Several experiments were conducted during the eclipses in Greece and Brazil, but few results have been published, and except for the analysis of Kane[6] for the May 1966 eclipse in Greece, a detailed interpretation of the ionization changes has not yet appeared in the literature. The limitations of the ground-based data and the transitory nature of the rocket data are such that the maximum of the eclipse effect, and the time at which the maximum effect occurred with respect to the visible solar eclipse, are not very well established.

The eclipse of March 7, 1970, provided the opportunity of obtaining detailed data concerning the changes of electron density in the lower ionosphere using the somewhat sophisticated two-frequency differential absorption-partial reflexion systems at Ottawa[9]. Because the eclipse at Ottawa was not total (77·8 per cent at 80 km), the observations were supplemented by obtaining partial reflexion data, but with a lower performance system, at a location on the east coast of Canada, in Nova Scotia, where the eclipse was total. The observations in Nova Scotia were supplemented by *in situ* rocket measurements.

A temporary launch site was set up near East Quoddy, N.S. (44° 54·4′ N, 62° 20·1′ W). The geographic (and magnetic) latitude of the launch site is similar to that for Ottawa. A partial reflexion sounder using a four-dipole antenna system was installed at the launch site.

The rockets were launched at an azimuth of 146°, perpendicular to the eclipse track, at an elevation angle of 85°. Apogee, about 150 km, was near the centre of the midpath of the eclipse track at this altitude. Four Black Brant III rockets were launched: the first (rocket AAF-III-42) just before first contact, at 13:40:41 AST; the second (AAF-III-43) at 14:55:41 AST, so that the vehicle would be in the eclipse shadow from ground to apogee (Fig. 1): and the third and fourth (AAF-III-44 and AAF-III-45) two and eight minutes after the launch of the second rocket. The particular launch sequence was chosen because observations during previous eclipses by long wave propagation (unpublished work of Belrose) have shown that the most rapid changes occur near totality, and that the maximum of the eclipse effect is delayed after the maximum of the visible eclipse by six minutes.

The rocket-borne experiments were designed to measure simultaneously electron and ion density, by a propagation experiment and by Langmuir probes, and the intensity of pertinent ionizing radiations, by measuring the intensity of atmospheric absorption of solar X-radiation (1·5–8 Å) and of Lyman alpha radiation. The ground-based partial reflexion experiments provide (1) control day data to establish that the eclipse day was normal or otherwise, (2) data on the regular diurnal change so that this can be allowed for in establishing the change associated with the eclipse, and (3) a detailed measure of ionization changes throughout the eclipse for comparison with the transitory data obtained by rocket-borne experiments.

This research was supported by the Space Research Facilities Branch of the National Research Council and by our individual institutions. We thank many col-

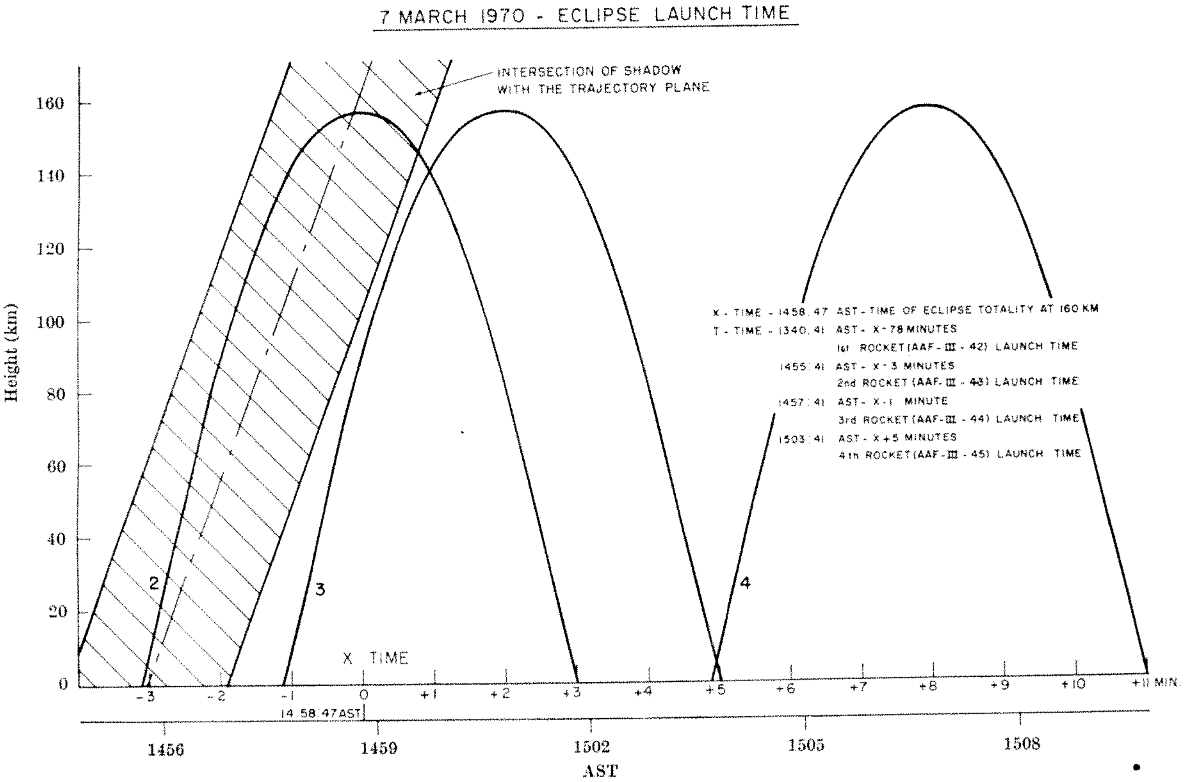

Fig. 1. Predicted trajectories with actual rocket launch times for the three rockets launched near totality. The shaded area is the intersection of the shadow with the trajectory plane.

leagues in the participating groups for help. The success of the eclipse rocket launches was made possible by the outstanding contribution of many; in particular Bristol Aerospace Ltd, in Winnipeg, the Department of Transport Meteorological Services, and the Department of National Defence Maritime Command and the Department of Transport Marine Services who provided range surveillance.

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(2) Radio Propagation Experiments

GROUND-BASED and rocket-borne radio wave propagation experiments were used to study ionization changes during the solar eclipse of March 7, 1970, and the diurnal variations on control days. The eclipse path passed along the eastern coasts of the United States, and Nova Scotia and Newfoundland in Canada. The path of totality was only some 140 km wide, but a large part of the United States and Canada was partially eclipsed, and the visible eclipse in the mesosphere (at 80 km) over Ottawa was 77·8 per cent.

Observations on eclipse day, and on control days before and after the eclipse, were made by the partial reflexion method at East Quoddy (where the eclipse was total) and at Ottawa, Ontario. The observations at East Quoddy were for a 10-day period centred on the eclipse day and restricted (primarily) to the same times of day as when the eclipse occurred on the eclipse day. Observations at Ottawa were from dawn until dusk on each day, from November (as part of our studies of the winter variability) to March 1969/70.

The rocket-borne propagation experiment consisted of reception in the vehicle of the 2·66 MHz circularly polarized pulsed transmissions from the ground-based partial reflexion sounder. The receiver output provides a measure of attenuation of each of the two magnetoionic components, and hence an instantaneous measure, independent of rocket aspect and spin, of the differential attenuation.

So far the partial reflexion data at Ottawa (at both 2·66 and 6·275 MHz) and at East Quoddy at 2·66 MHz have been analysed, and preliminary electron densities for all four rocket flights have been determined. The partial reflexion data at East Quoddy were contaminated to some extent by radio interference (a marine radio telephone frequency, rather close to the operating frequency, was intermittently in use at the time of the eclipse), but portions of the data over the period of the eclipse are quite good. The rocket propagation data have been scaled from paper chart records made at the launch site, and analysis of data reproduced more accurately in the laboratory from magnetic tape recordings is currently in progress. This letter presents some of the Ottawa data, and discusses the first-look results obtained at East Quoddy.

The chief features of the partial reflexion experiment have been described already[1,2]. A high power (100 kW) pulsed MF or HF radio wave is used to probe the ionosphere. The installations used for the eclipse observations are described in Table 1.

Table 1. STATIONS USED IN THE PARTIAL REFLEXION EXPERIMENT

Location	Frequency (MHz)	Transmitter power (kW)	Antenna	Beam width	Approx. beam power (MW)
Ottawa	2·66	350	40-dipole	28°	70
Ottawa	6·275	100	128-dipole	14°	72
East Quoddy	2·66	100	4-dipole	50°	1·6

Alternate left- and right-handed circularly polarized waves (O- and X-modes) are transmitted in quick succession every second, and the weak partial reflexions from the D-region are received on a circularly polarized antenna

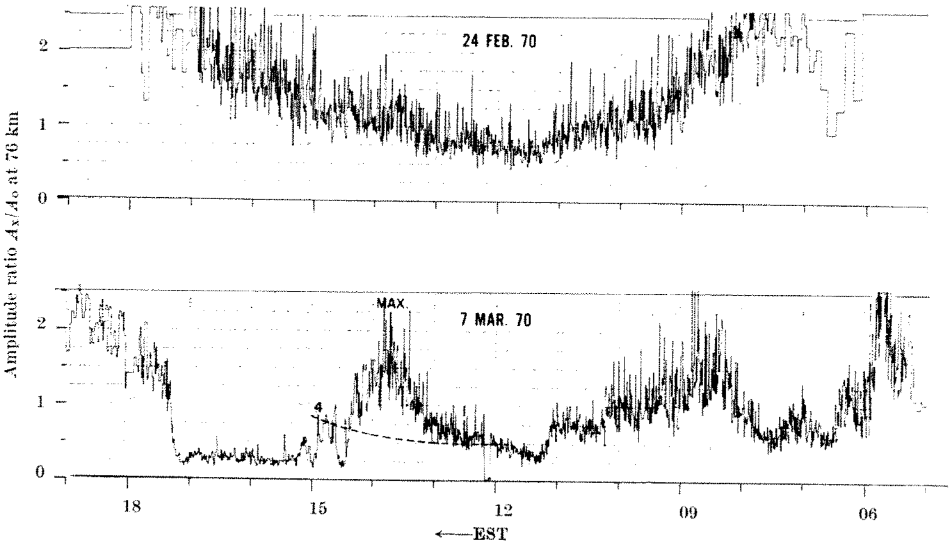

Fig. 1. Ratio-meter record (the A_X/A_0 ratio at 76 km) at Ottawa at 2·66 MHz for an undisturbed day (February 24, 1970) and for the eclipse day (March 7, 1970). The dashed line in the lower record is intended to represent the background level in the absence of the eclipse.

system, and amplified by a sensitive receiver. The basic experimental parameter is the relative amplitude of the O- and X-mode backscattered from the D-region measured as a function of height. The analogue voltage (height against amplitude echo envelope) at the output of the receiver is converted into a digital form and a digital value for each height interval of 2 km in a height range of 40 km is stored in a memory. This is done for both O- and X-mode waves. The information is then recorded on an incremental magnetic tape recorder, in a format compatible to computer analysis.

The rate of change of A_X/A_O with height, say in a 2 km interval, is a function of the change in differential absorption of the wave over that height interval, and thus, providing the collision frequency is known, the electron density can be determined. The collision frequency is determined from a measure of A_X/A_O at heights where differential absorption is negligible, and these values are extrapolated to higher heights using an assumed scale-height for the atmosphere. The A_X/A_O ratio at a particular height is inversely proportional to the electron density below that height, and is a very useful absorption parameter to study diurnal and interdiurnal changes. The ratio at a particular height can of course be determined after analysis of the recorded data on magnetic tape, but there was an urgent need to provide a record of this ratio which could be viewed and recorded in real time. Such a record could be used as a guide to inform the operator of the partial reflexion experiment when an ionospheric disturbance occurred; or in synoptic studies, because the prime data are recorded on magnetic tape, a ratio-meter record provides an invaluable aid to the subsequent more detailed analysis by computer. A digital value for each 2 km height is available in the memory each second, and so in principle it was possible to obtain the ratios of these binary numbers. There is considerable scatter in the instantaneous ratio[3], so the instantaneous values must be averaged in some way. The method developed (Ohno, private communication) involves the averaging of ten ratios, for which signal-to-noise was adequate, and of converting this value to an analogue voltage which is applied to a milliampere pen recorder. The pen recorder is held at this level until another ten good ratios have been recorded. Records obtained in this way at Ottawa are reproduced in Fig. 1.

The 2·66 MHz circularly polarized pulsed signals from the partial reflexion sounder at the East Quoddy launch site were monitored by a pulse receiver in the rocket. Pulses 50 μs long, at a rate of 20 s^{-1}, were transmitted with alternate left- and right-hand circular polarization (designated as O-mode and X-mode respectively). Positive identification was established by a differential in the spacing between pulses. The received amplitudes of each individual pulse were telemetered to the ground after logarithmic compression to a dynamic range of 40 dB. The pulses after peak detection were stretched to 5 ms so that they could be transmitted over a 200 Hz subcarrier channel of the telemetry, and in doing so the receiver was effectively gated off for this period; that is only the direct pulses and not the ionospheric reflected pulses were received.

The receiving antenna was a small ferrite loop mounted near the tip of the rocket and exposed after deployment of the nose cone. Rocket aspect and spin were not important, provided the rocket axis was approximately vertical, because even if there were spin modulation on the O- and X-wave amplitudes, the instantaneous amplitude ratio is not affected.

Solar activity increased and decreased regularly between November and February 1969/70; the 27-day periodicity in solar activity was pronounced. The 10 cm solar noise flux was a minimum on October 14–17, November 4–12, December 8–11, 1969, and January 6–9 and February 3–6, 1970. It was anticipated that there would be a minimum in solar activity just before the eclipse day, centred on approximately March 5. The 27-day periodicity, however, disappeared about the middle of February, and the Sun was very active, producing many solar X-ray (SXR) flares during the 10-day period before the eclipse, and a large geomagnetic storm ($A_p = 149$) occurred on March 8, 1970. Magnetic activity for several months before this date was very low ($A_p < 30$ during January and February). The direct and after-effects of this geomagnetic storm lasted for a week or more following March 8.

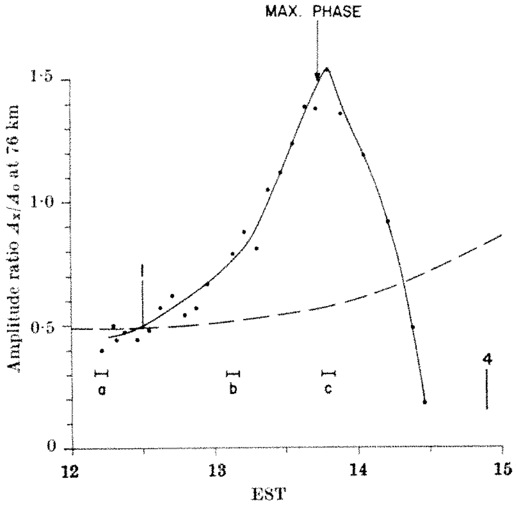

Fig. 2. Smoothed $(A_X/A_O)_{76\ \mathrm{km}}$ curve showing the solar eclipse effect observed at Ottawa at 2·66 MHz. The curve is drawn through the points averaged by computer in time intervals of five minutes, from the data recorded on magnetic tape.

The effects of these solar-terrestrial disturbances are well revealed by the ratio-meter records $(A_X/A_O)_{76\ \mathrm{km}}$. The periods of solar activity and the geomagnetic storm after-effects make it difficult to establish the regular variation for the period of one week before and one week after the eclipse, as well as the background on the eclipse day itself. The earliest date before the eclipse on which the regular diurnal variation can be observed in the absence of disturbance is February 24/25, 1970. The ratio-meter record for February 24 is shown in Fig. 1 (top). There is a clear diurnal change with the minimum ratio (that is, the maximum in electron density below 76 km) near local noon. Fig. 1 also shows the contrasting irregularity of the record for March 7, 1970. There was a marked disturbance (decrease in ratio) from 06–08 EST, which is probably a high energy particle (HEP) event; there was an SXR flare effect between 1106 and 1200 EST; and another and larger HEP event started abruptly at 1420 between the maximum phase of the visible eclipse and fourth contact. This latter event lasted until 1720 EST—what looks like a disappearance of the disturbance between 435–1510 EST is due to oblique reflexions. This is evident on examination of the A_X/A_O versus height data.

The background ionization, against which the eclipse effect is measured, is therefore rather difficult to establish. The dashed curve, in the lower record of Fig. 1, is intended to represent the variation that might have occurred in the absence of the eclipse. This curve has been fitted to the A_X/A_O values in the period just before first contact, and is extrapolated assuming a direct dependence on cos χ, where χ is the solar zenith angle. The dependence of the ratio on cos χ was established from observations on relatively undisturbed days before the eclipse.

The record of the ratio-meter is rather ragged as the trace is the average of only ten amplitude ratios. The curve in Fig. 2 shows a smoothed version of the ratio $(A_X/A_O)_{76\ \mathrm{km}}$ drawn through points averaged in time intervals of five minutes from the data recorded on magnetic tape. This curve shows quite clearly, as anticipated from LF propagation observations during previous eclipses, that the maximum of the eclipse effect was delayed (by about four minutes) after the maximum of the visible eclipse.

The partial reflexion observations at the rocket launch site near East Quoddy showed a very similar effect. The maximum of the effect was clearly delayed after totality, but the details of the curve for just before second contact cannot be established with any certainty due to radio interference from a nearby marine radio telephone channel.

The radio propagation experiment yielded good data on all four rocket launches. The first rocket was launched just before first contact when the solar zenith angle $\chi \approx 50 \cdot 4^\circ$. Differential absorption of the two magnetoionic components of the 2·66 MHz radio wave began at about 71 km altitude on this flight. The second rocket was launched during totality ($\chi \approx 61^\circ$) and the third and fourth rockets were launched two and eight minutes after the launch of the second rocket. On the three flights near totality differential absorption began at about 80 km. On the first flight the extraordinary (χ) wave was totally absorbed at about 86 km, and on the other flights near 102 km. The ordinary (O) wave was totally absorbed above 99 km on the first flight but continued to be received throughout the other three flights.

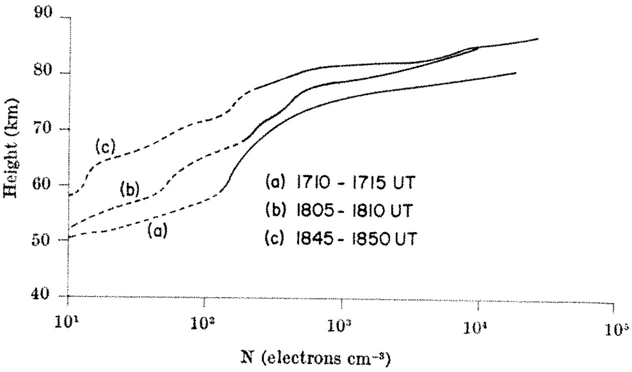

Fig. 3. Curves of electron density against height for Ottawa as determined by the two-frequency partial reflexion experiments showing the effect of the solar eclipse. The times for the various curves (labelled (*a*), (*b*) and (*c*)) are marked in Fig. 2.

The differential absorption partial reflexion data at Ottawa for both 2·66 and 6·275 MHz were analysed to yield height profiles of electron density at intervals of five minutes during the eclipse. Fig. 3 shows the profiles for 1710–1715 UT (EST plus 5 hours) which was just prior to first contact, for 1805–1810 UT when the visible eclipse obscuration was about 37 per cent, and for the time period centred on the maximum of the eclipse effect, 1845–1850 UT. Maximum obscuration over Ottawa at 80 km was 77·8 per cent. The profiles are labelled (*a*), (*b*) and (*c*), and the corresponding periods in time are indicated on Fig. 2. The continuous portions of the curves are based on the measurement of differential absorption at both frequencies, and the dashed extension to low heights is based on the assumption that in the height region where differential absorption is negligible the electron density is proportional to the O-wave amplitude[1].

Fig. 3 shows that there was a clear decrease in the electron density at all heights, the magnitude of which increased with decrease in height. The ionization densities at 80, 70 and 60 km changed approximately by factors of 2·5, 5 and 10 respectively. The results are much less reliable at the lower heights because of the extrapolation already mentioned.

The partial reflexion and rocket transmission data obtained at the launch site near East Quoddy have been analysed in a preliminary way to determine electron densities. The conclusions that can be drawn at present are as follows. The electron densities measured by the ground-based partial reflexion method and by the rocket transmission method are in reasonable agreement and indicate, as would be expected, a larger change than observed over Ottawa. The electron density at 80 km, for example, decreased by about a factor of 7. This can be contrasted with the total solar eclipse results of Kane[4], for the May 20, 1966, eclipse in Greece, which showed that the electron densities at heights of 85, 80 and 75 km changed by factors of about 7, 7·7 and 20.

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(3) Probe Experiments

Three Langmuir probes for the direct measurement of charge densities[1] were mounted on each rocket, at the forward end of the payload. These were protected by a split nose cone shroud which was released at 43 s into the flight, corresponding to an altitude of about 57 km. Probe No. 1 was mounted on the vehicle axis at the foremost tip of the payload. Probes No. 2 and No. 3 were mounted on arms 33 cm long which swung outward when the shrouds were released (Fig. 1). Probes No. 1 and No. 3 were spheres 0·635 cm in diameter, and probe No. 2 was 6·35 cm in diameter. All spheres were rhodium plated. With the exception of the fins, the entire rocket surface was clean metal to provide the maximum area for the return path of the current collected by the bipolar probe systems. A sufficiently large ratio of rocket body area to probe area is required to prevent distortion of the probe-to-plasma voltage sweep, and to prevent interaction of the probes through perturbation of their reference potential. These rockets yielded an area ratio of 30,000 for the small probes and 300 for the large probe. The effectiveness of the area ratio was checked in flight by searching for interaction of the asynchronous sweeps in the current characteristics of the probes. No interaction was detected in the flight records.

Two separate and asynchronous voltage sweeps were used; one was applied to probe No. 1 and the other was applied in common to probes No. 2 and No. 3. These waveforms are shown in Fig. 1. The sweeps contained intervals of constant potential for the examination of the time variation of positive ions and electrons, and a linear sweep interval for the examination of the behaviour of the accelerating and retarding potential. The dual asynchronous sweep system and the multiple probes had the following assets: continuous recording of the spatial variation by at least one probe at any time, greater dynamic range of current collection, examination of the effect of probe size and a resulting increase of confidence in the calculated densities, and a direct check on the effectiveness of the bipolar probe area ratio in maintaining a stable reference potential.

The sweep rate, sweep cycle period, and the rocket roll rate were selected to obtain adequate height resolution, to minimize the effect of roll modulation, and to keep the signal variations within the capability of the telemetry

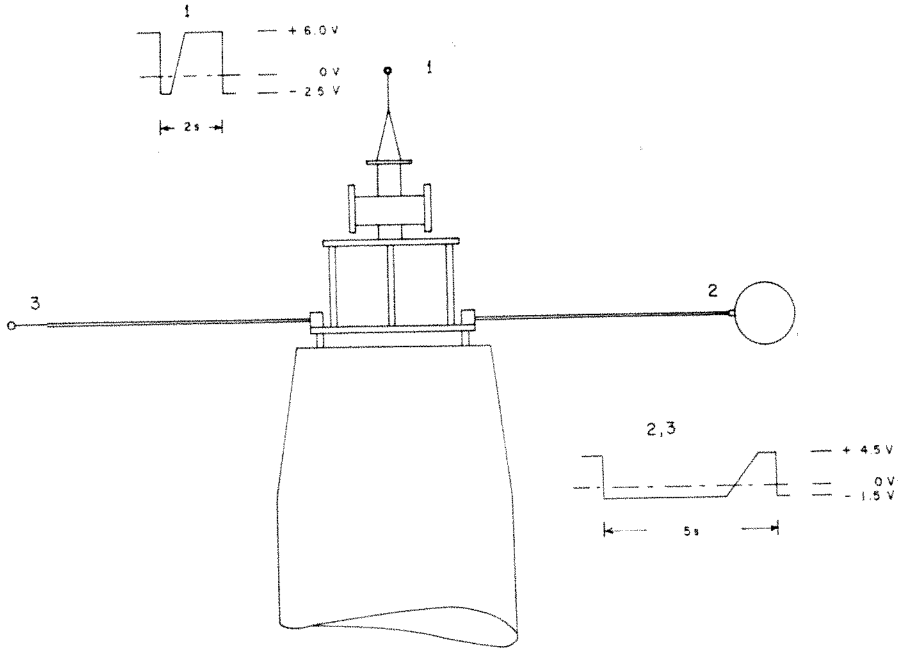

Fig. 1. In-flight configuration of the Langmuir probes. AAF-III-42/45.

bandwidth. The periods of the sweep cycles were 2·0 and 6·0 s respectively.

In addition to the Langmuir probes, a simple phototransistor photometer looking sideways was flown to monitor the visible light illuminating the rocket. This experiment also provided backup and auxiliary information for the much more accurate optical aspect sensing experiment described in the following paper.

Magnetic aspect was obtained from a three-component magnetometer system included in the payload.

The rockets were spun up to 8 Hz to obtain good stability, and then were despun at 36 s after launch to a planned roll rate of 0·6 Hz. The final roll rate was chosen to match the design values of the experiments.

Of the twelve Langmuir probes flown on the four rockets, all operated successfully except for the 6·35 cm sphere on the first rocket, AAF-III-42. This probe broke early in the flight and no data were obtained from it, but data from the other two probes on this rocket were good. The despin mechanism on this rocket failed to operate properly and the final roll rate was 1·4 Hz. This roll rate was within the tolerances of the experiments, however, and no degradation of the data occurred.

Complete reduction of the probe characteristics to charge densities has not been made yet, but plots of the currents collected by the probes give a good indication of the general behaviour of the ionization. Data were obtained from probe deployment at about 57 km to apogee at approximately 150 km. In all flights, a distinct peak in the E-region at 106 km altitude was observed. The peak electron densities during the pre-eclipse launch of AAF-III-42 at 1341 AST were typically about three times greater than those during the period around totality. Significant and rapid changes were observed in the ionization profile during the rapid sequence of launches around totality. Further details must await the complete scaling and reduction of the data from all the probes.

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(4) Observations of Solar Ionizing Radiations during the Eclipse

The intensity of solar radiation at the Lyman-α wavelength, 121·6 nm (1216 Å), and in the part of the X-ray spectrum from 0·15 nm to 0·8 nm (1·5–8 Å) was measured immediately before and during the solar eclipse by experiments carried on the four Canadian Black Brant III rockets launched from East Quoddy, Nova Scotia. The purpose of the experiments was to provide data on the change in the production rate of ionization in the D-region of the ionosphere during the eclipse, so that it can be related to the change in the distribution of ionization measured by other experiments in the same payload. The first rocket was launched just before first contact, the second at totality and the third and fourth at two and eight minutes later. The trajectory of flight 2 combined with the movement of the eclipse kept the rocket in the umbra from launch until apogee. The eclipse was partial during the second half of the flight and during the whole of flights 3 and 4.

The Lyman-α sensor was an ionization chamber of coaxial construction with an end window. An effective wavelength bandpass was formed by the transmission cut-off of the lithium fluoride window and the ionization potential of the nitric oxide gas filling. The sensor was triaxially connected to a current amplifier through a −45 V supply on the central electrode. To minimize leakage current the guard electrode was also biased to −45 V. The amplifier produced a five volt output for a 20 nA input current. Three cascaded amplifier stages increased the overall sensitivity to about 20 pA.

The X-ray experiment was contained within two packages; one housed the proportional counter detector and the stabilized bias supply and the other the amplifier, pulse handling and counting circuits. The detector, which was designed by Leicester University, was of pill-box shape with a diametric anode wire and a 4 cm^2 window of beryllium, 0·013 cm thick. A collimator restricted the transverse angular response to ±30°. Operated at a bias level of 1600 V the counter had a gas gain of about 2×10^4. The spectral range of the counter was set by the thickness of the window and the pressure of the krypton filling. X-ray photons which stopped in the counter each produced

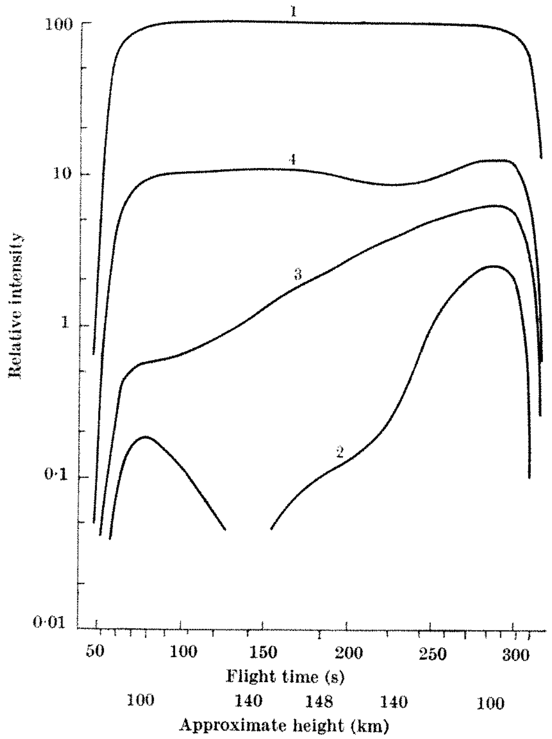

Fig. 1. **Solar Lyman-α intensity normalized to the full Sun intensity observed at apogee on flight 1. Curve 1, the intensity observed by flight 1 launched 75 min before totality; curve 2, the intensity observed by flight 2 launched at totality; curve 3, the intensity observed by flight 3 launched 2 min after totality; curve 4, the intensity observed by flight 4 launched 8 min after totality.**

a charge pulse with amplitude proportional to the energy of the incident photon. These pulses were amplified to form proportional voltage pulses. A series of voltage comparators and gating circuits were used to sort the pulses into seven intervals corresponding to predetermined wavelength ranges.

The intensity, I, of Lyman-α radiation is related to the current produced in an ionization chamber by

$I = 1{\cdot}025\times10^5\dfrac{i}{\eta A(\theta)}$ (J m^{-2} s^{-1}) where i is the chamber current in amps, η is the chamber efficiency (ion pairs per normally incident photon), $A(0)$ is the effective window area (m^2), and θ is the angle of incidence. The rockets were spin stabilized and the window faced the Sun approximately once per second. Fig. 1 shows the observed peak Lyman-α intensity as a function of flight time for the four rockets. An approximate height scale is shown which is reasonably accurate for the uplegs of the flights. The curves have been normalized to the intensity of the full Sun at the time of apogee of flight 1. Curve 1 from 50 to 80 s shows an increase in intensity as the first rocket ascended into the less dense atmosphere. This variation can be used to determine the molecular density of oxygen in the mesosphere using laboratory values of absorption coefficient. Over the central part of the flight, the intensity was essentially constant because atmospheric absorption of Lyman-α is negligible above 100 km. After 280 s absorption became significant again and the Lyman-α intensity decreased. During the first half of flight 2, while the rocket was in total shadow, the intensity never rose above 0·2 per cent of the full Sun value. This is a significant signal, however, and must have come from outside the disk of the Sun, but this does not necessarily mean that there were any sources of Lyman-α outside the disk because hydrogen in the outer atmosphere of the Sun could have scattered the radiation. The second half of flight 2 showed an increase in Lyman-α as the rim of the Sun appeared. Apart from atmospheric absorption effects, the intensity increased monotonically throughout flight 3 as more of the Sun became uncovered. The last flight showed that the intensity had recovered to about 12 per cent of the full Sun value at ten minutes after totality. Neither these nor the X-ray results have been corrected for any departure of the rocket attitude from the expected behaviour, but no large corrections are anticipated.

At times when the sensors were facing away from the Sun due to rocket spin, the Lyman-α intensity did not fall to zero. Fig. 2 shows the background intensity observed during flights 1 and 3. The background intensities measured on flights 2, 3 and 4 were similar and about a third of that measured on flight 1. Unlike the signals from the Sun the background Lyman-α continued to increase in intensity up to the apogee of the trajectory. This is probably because the background, due to resonant scattering of Lyman-α by hydrogen in the Earth's atmosphere, entered the sensor from a large range of zenith angles. The relatively small difference between the full Sun and eclipse backgrounds supports this view because, in the latter case, scattering must have been from distant parts of the Earth's atmosphere still strongly illuminated by the Sun.

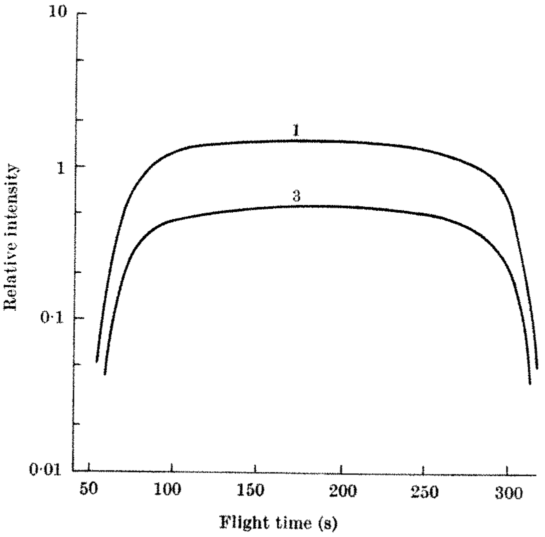

Fig. 2. Background Lyman-α intensity normalized to full Sun intensity. Curve 1, background intensity observed on flight 1; curve 3, background intensity observed on flight 3.

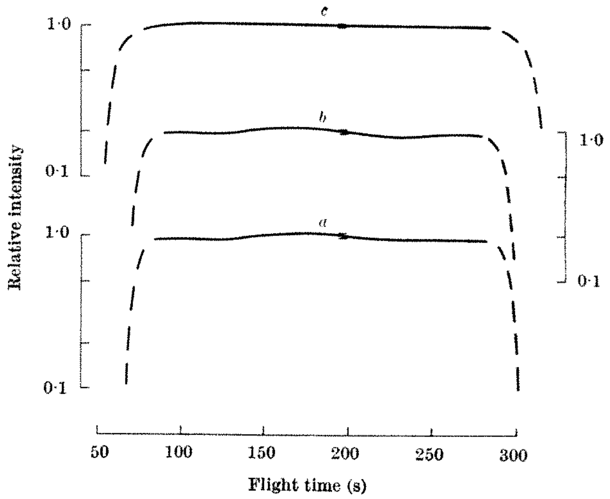

Fig. 3. Comparison of normalized X-ray (*a*, 0·25 nm; *b*, 0·6 nm) and Lyman-α (*c*) profiles, flight 1 (full Sun).

The X-ray photon count was recorded in seven channels covering energies corresponding to wavelengths from 0·15 nm to 0·8 nm. Only the data from channels 2 and 6 which centred on approximately 0·6 nm and 0·25 nm repectively will be considered here.

As the rocket rotated on its axis, the X-ray sensor faced the Sun approximately once per second. The counts observed during each rotation were recorded and a small background count has been subtracted. Fig. 3 is a plot of the count rate for channels 2 and 6 observed on flight 1, each normalized to the maximum count observed during the flight, and the Lyman-α curve from Fig. 1 is shown for comparison. The parts of the curves severely affected by atmospheric absorption are shown broken.

Analogous curves for flight 2 are given in Fig. 4. The variation in the X-ray intensity in both channels is superficially similar to the Lyman-α variation, but there are differences in detail. The Lyman-α intensity increased by a factor of ten after 220 s, whereas both X-ray channels had their maximum rate of increase earlier than this, at a time when the solar disk was still covered. The differences in the shapes of the curves are significant, and the error bars indicate the random spread in the individual points, derived from the counts per rocket rotation.

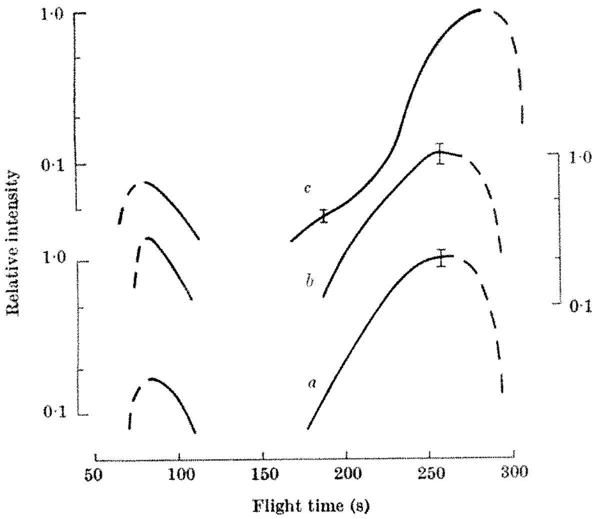

Fig. 4. Comparison of normalized X-ray (*a*, 0·25 nm; *b*, 0·6 nm) and Lyman-*α* (*c*) profiles, flight 2 (at totality).

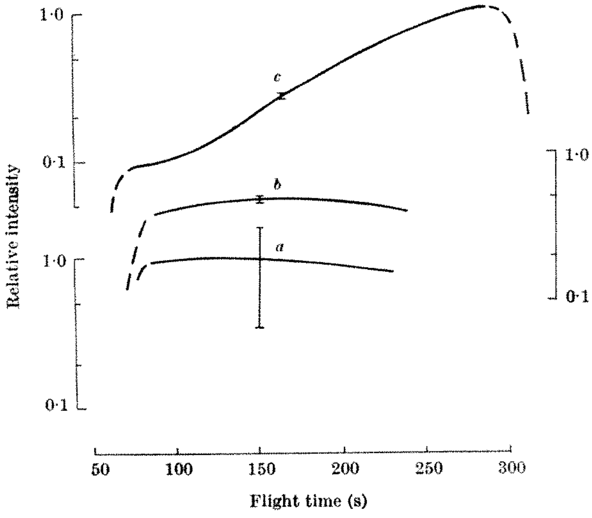

Fig. 5. Comparison of normalized X-ray (*a*, 0·25 nm; *b*, 0·6 nm) and Lyman-*α* (*c*) profiles, flight 3 (2 min after totality).

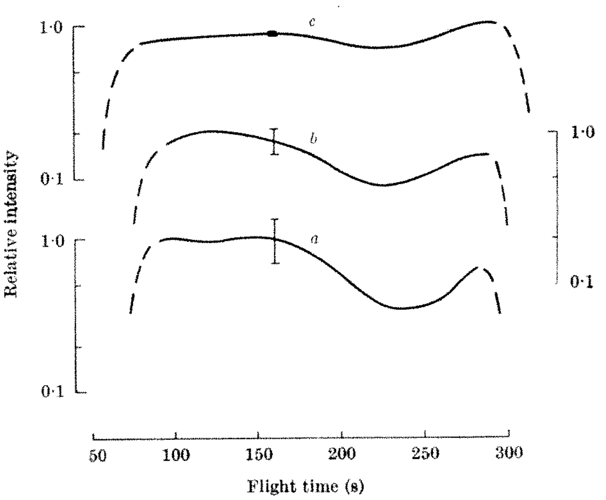

Fig. 6. Comparison of normalized X-ray (*a*, 0·25 nm; *b*, 0·6 nm) and Lyman-*α* (*c*) profiles, flight 4 (8 min after totality).

The data from flight 3 presented in Fig. 5 again show differences between the intensity variations for Lyman-α and X-rays. The former increased throughout this flight as the Sun became uncovered, whereas the latter was fairly constant, actually decreasing a little after a flat maximum near 150 s. This decrease is not too difficult to explain because X-rays usually originate in a few small regions of the Sun but the source of Lyman-α is from much larger and more widely distributed patches. Although the area of uncovered Sun increased through the flight, the orientation of the exposed crescent changed due to the motion of the rocket, and it is quite possible for a small X-ray source to have become covered during the later part of the flight. It has not yet been possible to analyse the X-ray data for flight 3 after 240 s.

Fig. 6 shows the data for flight 4. In this case, the intensities of X-rays and Lyman-α did not change much during the flight, which means that there were no new major sources uncovered. The dip at about 230 s probably corresponds to an active region becoming partly covered and then uncovered again. The decrease was much more significant for X-rays than for Lyman-α.

The solar radiation data from these four rocket flights will be used to calculate the production of ionization in the D-region as well as to study the sizes and distribution of Lyman-α and X-ray sources on the Sun. The absorption of the radiation in the lower atmosphere will be used to find the atmospheric density. The Lyman-α intensity will be combined with data on nitric oxide concentration, obtained by other workers, to calculate the distribution of production of ionization. The data from the seven X-ray channels will be used to derive an X-ray spectrum. Combining this with the known absorption and ionization cross-sections, the production of ionization due to X-rays will be calculated and added to that due to Lyman-α. A study of the relation between variations in the production and the density of ionization during the eclipse should give information on loss rates and possibly other ionospheric parameters.

We acknowledge the parts played by our colleagues in obtaining these data, and thank the National Research Council of Canada for inviting us to take part in its eclipse programme.

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Preliminary Results from a Meteorological Rocket Experiment

THERE is little question that important ionospheric changes occur during a solar eclipse. Dramatic evidence was obtained, for example, in the eclipse of November 12, 1966, when rocket measurements in Brazil showed a reduction in the electron concentration of the D-region by several orders of magnitude, symmetrical about the time of totality (unpublished report). Less secure is the evidence for changes in the neutral stratosphere and mesosphere. Large increases in the amount of total ozone deduced from ground observations are at variance with the increases deduced theoretically by Hunt[1], who believes that neglect of limb darkening of the Sun would have influenced the past results obtained with Dobson spectrophotometers. For meteorologists the temperature of the upper stratosphere, determined initially (in the absence of circulation effects) by solar absorption by ozone in the Hartley region, is of primary importance. Temperature changes due to an eclipse and those occurring from day to night are not exactly comparable, because in the latter case there is a gradual but large variation of solar elevation angle. It is nevertheless interesting that satisfactory agreement has not been found (unpublished work of Quiroz) between observed day–night temperature changes and theoretically derived estimates of the diurnal variation[2].

The changes occurring during a total solar eclipse are of special interest because of the rapid obscuration of the Sun at a relatively constant solar elevation. Provided that the real temperature variation can be separated from instrumental and radiational error, the eclipse data may throw light on the magnitude of the radiational error and perhaps also on the real diurnal variation. The first rocket attempt[3] to find the temperature change due to an eclipse was made on November 12, 1966, at Tartagal, Argentina (23° S, 64° W). Although a detailed analysis was not published, the observational data indicated a temperature drop exceeding 10° C near 50 km, in the eclipse, and an increase of about 20° C at 60 km from before totality (1034 local time) to an observation time in mid-afternoon. Clearly some clarification of the temperature changes due to an eclipse is needed. Moreover, a large temperature variation if sustained over a deep atmospheric layer would imply a large perturbation in the pressure field, with significant effects on the circulation. Indeed, Chimonas and Hines[4] have recently suggested that measurable internal gravity waves should be induced by the cooling action of an eclipse.

An experiment with small meteorological rockets was thus carried out at Wallops Island, Virginia. (37° 50′ N,

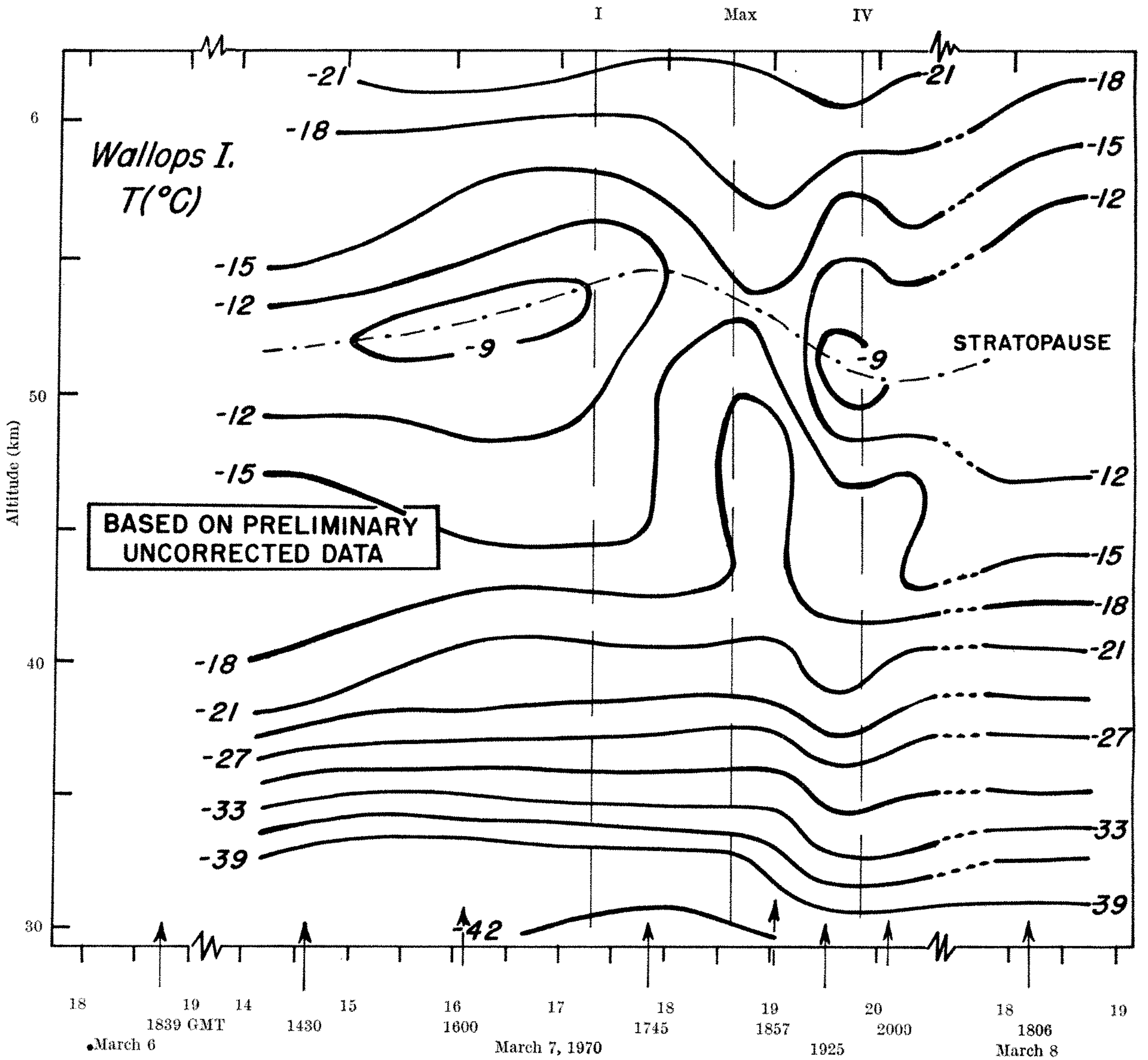

Fig. 1. Preliminary analysis of smoothed rocketsonde temperatures for Wallops Island, Virginia, for the solar eclipse of March 7, 1970 indicating a temperature decrease by as much as 7 degrees within the layer 40–60 km.

75° 29′ W), giving detailed temperature and wind data before, during, and after the eclipse of March 7, 1970. Wallops Island was ideally suited for this purpose because the expected trajectory of the sensors would place them close to the central path of the eclipse[5]. The purpose of this report is to describe the experiment and to present a preliminary analysis of the temperature results.

Table 1. ARCAS ROCKET SOUNDINGS AT WALLOPS ISLAND

Launch date	Time (GMT)	Rocket apogee (km)	Highest alt. of temp. data (km)	Sensor fall rate (m s^{-1}) 60 km	50 km	40 km
Mar. 6	1839	52·1	49·8		70	35
Mar. 7	1430	62·9	61·1	117	80	36
	1600	67·1	62·3	117	76	33
	1745*	66·8	63·9	125	68	34
	1857*	64·0	61·2	117	68	34
	1925*	66·1	63·6	181	80	34
	2000	64·5	61·6	142	68	35
Mar. 8	1806	67·4	60·0	142	71	33

Primary tracking by FPS-16 precision radar, except for observation at 1857 (MPS-19 radar).

* Eclipse sounding.

Eight ARCAS rockets were launched at the times given in Table 1. Three of these were fired during the eclipse, which began at 1720 and ended at 1952 GMT, with maximum (complete obscuration) at 1838 GMT (1338 EST). The remaining ARCAS soundings served to establish the background meteorological conditions. From the standpoint of altitudes attained and data acquisition the experiment was eminently successful. Minor disappointments were a relatively low apogee on the day before the eclipse, and a broken thermistor in a sounding originally scheduled for 1745 GMT on March 8. The latter required the launching of a backup ARCAS rocket 21 minutes later (1806 GMT). Radar acquisition and telemetry were excellent throughout. A series of LOKI rockets, less sensitive to wind, were made ready for launching in the event of very strong low level winds, but these were not required.

Obviously it would have been desirable to fire one or two more small rockets close to the time of totality, but logistical requirements for the total complex of experiments conducted at Wallops Island on March 7 precluded this. We look forward to the review of temperatures from rocket soundings with pitot static probes by the Goddard Space Flight Center and the University of Michigan; these measurements obtained at 1759, 1826, and 1841 GMT during the eclipse are free from any radiation error and should therefore serve as a further check on our results. Our final analysis will not only take these data into account, but will also be made within the context of the pattern of ozone variation measured by NASA.

A direct analysis of the temperature data showed much small-scale detail of the type commonly found in atmospheric soundings[6]. To portray the main features, averages over 3 km overlapping by 1 km (thus yielding smoothed temperatures at 2-km intervals) were obtained. These were analysed objectively without special attention to the eclipse phase, and subsequently verticals were entered (Fig. 1) to denote the begining (I), maximum, and end (IV) of the eclipse. Arrows for observation times are 5 minutes after launch, corresponding to the time when the sensors descended to approximately the 50-km level. At this time and altitude the measurements during the eclipse were near a point 50 km (27 nautical miles) ESE of Wallops Island. At this geographical location and altitude, the obscuration of the Sun was 27, 64, and 21 per cent, respectively, 5 minutes after the eclipse launch times 1745, 1857, and 1925 GMT. For the preliminary analysis, the sounding on the day before the eclipse has been omitted until a temperature variation near 40 km, apparently of synoptic origin, is examined with the aid of data obtained earlier in the week.

Fig. 2*a* illustrates the detail present in the original data for two selected soundings and Fig. 2*b* shows the effect of smoothing the data. Note the meeting of the curves just above 60 km.

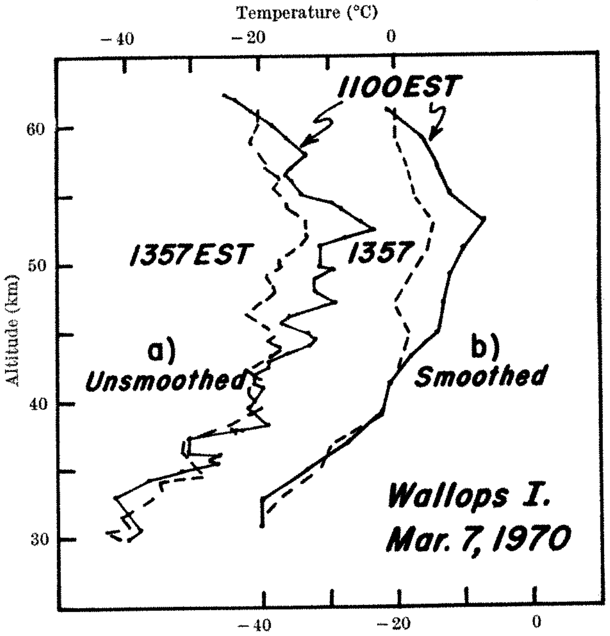

Fig. 2. Smoothed and unsmoothed temperature profiles for sounding before eclipse (1600 GMT = 1100 EST) and sounding during eclipse (1857 GMT).

In Fig. 1 the most arresting features are: (1) A perturbation in the temperature field essentially contained within the period of the eclipse, and chiefly within the layer 40–60 km. (2) Maximum amplitude 5°–7° C at 45–55 km, with minimum temperatures indicated a few minutes after totality. (3) Rapid warming just before the end of the eclipse, as indicated by the 1925 GMT observation. This effect, however, may be partly spurious. Recent comparison tests have shown good agreement between meteorological rocket soundings obtained a few minutes apart, under controlled conditions. To illustrate, the r.m.s. difference in each of several 5-minute pairs of LOKI-Datasonde temperature profiles was less than 2° C and similar results were obtained in pairs of ARCASonde profiles from rockets launched a few days before the eclipse experiment. To preclude an error contribution from the sensors, the bead thermistors were minutely examined and carefully recalibrated just before the experiment.

Under the influence of some direct radiational error and aerodynamic heating due to the fast descent of the sondes, however, the sensors give temperatures which above about 50 km may be several degrees above ambient. The radiational error has been considered small (unpublished work of Ballard), less than 3 degrees, but in any case should be examined anew according to the observational circumstances. In the present data the fact that cooling appears in a discrete layer, with relatively unchanged temperatures above as well as below, indicates that the perturbation is not due simply to radiational error, which would increase upward. Corrections for these factors will be applied by established methods to the observed temperatures (unpublished work of Lacey and Staffanson), but except for the variation imposed by the observation at 1925 GMT, the overall pattern should not be strongly affected. From Table 1 it is seen that the sensor at 1925 underwent an especially high rate of fall, so that aerodynamic heating greater than usual may partly account for the slope of the isolines around that time. For this preliminary analysis, however, the extra heating expected from the increase in fall rates from 1857 to 1925 GMT was evaluated numerically, and an adjustment was made to the curves above 50 km.

Final conclusions must await not only refinement and correction of the present data, but also comparison with other results, especially the pitot tube measurements at Wallops Island and meteorological results obtained on March 7 by experiments from White Sands Missile Range at Eglin Air Force Base, Florida, and in Mexico. An explanation of the difference in temperature results in 1966 at Tartagal and in the recent eclipse should be sought. Some of the difference may be due to the differing solar elevation at the time of maximum eclipse (about 70° at Tartagal and 40° at Wallops Island).

Wind data have not been completely processed. Pressure data derived hydrostatically from the March 7 temperatures indicate a variation in the height of the 0·4 mbar surface (the highest pressure level for which synoptic maps are now prepared) by about 300 m. Thus we may also expect to see some effect in the winds from the eclipse.

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Rocket Density Measurements at Eglin, Florida

THREE falling sphere experiments instrumented with omnidirectional accelerometers were launched at Eglin, Florida, during the total eclipse of March 7, 1970. The first rocket was launched near first contact, the second near totality and the third near fourth contact.

Recent observations of neutral atmospheric density and temperature obtained with the 7-inch falling sphere rocket experiment indicate that above 60 km the short-term variations of the order of 3 to 6 h are sometimes greater than those of seasonal average values. This applies in particular to the temperature results obtained near the level of the mesopause[1,2] and to density results between 90 and 105 km (ref. 3). In fact, the amplitudes of these variations are usually large enough to preclude accurate measurement of a diurnal effect in these altitude regions. Diurnal variations of the order of 7–20 per cent have been determined, however, from 30 to about 70 km (ref. 4 and unpublished work of Thiele). On the basis of these observations it seems reasonable to speculate that only small changes in density are likely to occur under the short-term transient conditions of a total eclipse. The three *in situ* measurements of neutral density reported here were therefore intended for use primarily as an accurate reference frame for correlating measurements of total density with measurements of O_2 and O_3 from optical payloads (conducted by L. Weeks, AFCRL) launched at Eglin Gulf Test Range and other ionization and composition experiments launched from Wallops Island, Virginia.

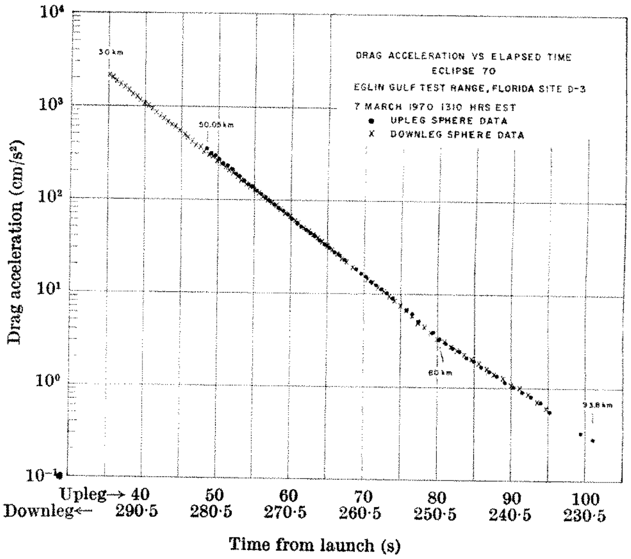

Fig. 1. Results from rocket 2 launched near totality.

Sphere trajectories and rocket sub-points (position coordinates and altitude as a function of time from launch) are being calculated at present to determine the positions of the measurements relative to the progression of the eclipse. Table 1 summarizes the launches.

The launcher azimuth settings, calculated for optimum interception with the path of the eclipse, were compromised slightly by high surface winds that prevailed during each firing. The preliminary flight results presented here consist of sphere drag acceleration data from which density profiles are determined.

The AFCRL 7 inch rigid falling sphere which determines density in the altitude range 30 to 120 km (refs. 5 and 6) was used on rockets 2 and 3 near totality and fourth contact respectively, and is instrumented with an omnidirectional accelerometer and telemetry. It is deployed from an ascending rocket at an altitude of 50 to 60 km. At payload separation the accelerometer begins to sense atmospheric drag on the sphere during its free flight. The data are telemetered to ground-based stations which record the information on magnetic tape and provide a digital print-out in real time. Temperature results are deduced from the density data. The system error is not greater than 5 per cent at the highest levels of the measurements (Murphy, unpublished).

The AFCRL 10 inch MICRO-G falling sphere, launched near first contact, was flown for the first time during this eclipse. It was instrumented with telemetry and a three axis ultrasensitive accelerometer which operates on the principle of a capacitive bridge. The voltage output from each of three orthogonal axes is approximately proportional to the displacement of a reference mass from a central or null position within a spherical cavity. The mass, which is positioned mechanically at the centre, is displaced by air drag on the exterior surface of the falling sphere. The accelerometer was developed by the Upper Air Research Laboratory of the University of Utah under an Air Force contract. Preliminary evaluation of the flight data indicates a threshold sensitivity of about

Table 1. LAUNCH SUMMARY OF FALLING SPHERE PAYLOADS, CAPE SAN BLAS, FLORIDA (LAT. 29° 40′ N, LONG. 85° 21′ W)

Rocket No.	Vehicle No.	Payload type	Launch time (EST)	Launch elevation (effective)	Launch azimuth (effective)	Rocket peak altitude (km)	Rocket peak time (s)	Height range of data (km)
1	A07·015–1	10-inch sphere w/tri-axial accel.	1151	85°	130°	195	200	30–150
2	A07·016–1	*a*, 7-inch sphere w/AFCRL omnidirectional accelerometer. *b*, Chemical release (NaLi)	1310	85°	220°	111	165	30–94
3	A07·016–2	7-inch sphere w/AFCRL omnidirectional accelerometer	1425	85°	134°	248	251	30–112

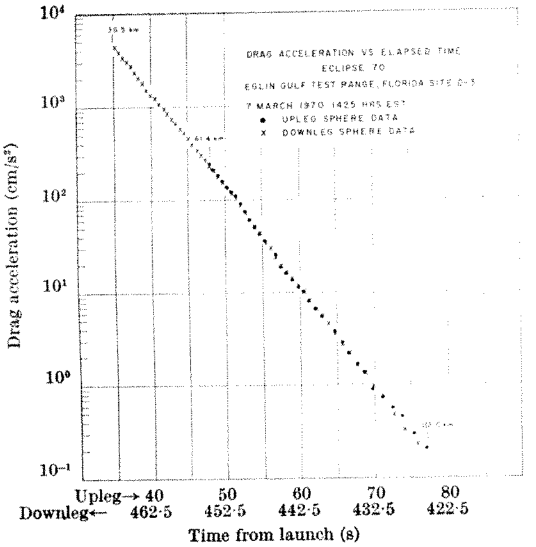

Fig. 2. Results from rocket 3 launched near fourth contact.

10^{-7} g and a useful altitude range of about 30 to 145 km when the payload is boosted by a Nike Iroquois rocket. Total drag acceleration is obtained from vectorial addition of the components of acceleration on each axis of the accelerometer.

The drag acceleration results shown in Figs. 1 and 2 were obtained from 7 inch falling spheres. Because drag acceleration is proportional to density the results presented here are useful for qualitative evaluation of the data.

The data of rocket 2 launched near totality, which attained a peak altitude of only 111 km due to a malfunction, are shown in Fig. 1. Drag accelerations obtained during ascent and descent are in excellent agreement, although the independent measurements were separated in time by approximately three minutes. Note that the downleg time scale is reversed so that both ascending and descending drag curves have similar slopes to facilitate comparison of the data. Because drag acceleration, like density, is a logarithmic function of altitude, a semilog plot of drag acceleration as a function of elapsed time (for selected intervals) should fall approximately along a straight line for a normal or undisturbed atmosphere, provided the trajectory is nearly symmetrical with peak time. The data in Fig. 1 show some structure above about 70 km which may be due to a departure from normal conditions. Because of the excellent agreement in ascent/descent data even the small perturbations observed must be considered as real changes in the thermodynamic structure of the atmosphere.

As noted in Table 1, a chemical release payload was combined with the falling sphere on rocket 2 to provide simultaneous upper atmospheric measurements. The objective of the chemical release experiment, proposed by K. S. W. Champion of AFCRL, was to measure upper atmosphere motions in the 80–150 km altitude range during the maximum phase of the eclipse. The payload consisted of sodium and lithium to be released in sequence. These chemicals were selected to obtain maximum contrast between the chemical trail and the sky background when photographed with appropriate interference filters on the cameras. Three camera sites, used to determine atmospheric motions by standard triangulation techniques, were located so as to be in darkness during totality while the chemical release trail would be partially sunlit. Unfortunately no observations were obtained due to heavy cloud cover.

The results obtained from rocket 3, launched near fourth contact, are shown in Fig. 2. These data also exhibit structure, but to a smaller degree than that observed on rocket 2. This seems consistent with the concept that data obtained at late times relative to the maximum phase of the eclipse should represent near normal atmospheric conditions.

Flight results obtained on rocket 1, launched near first contact, have been reduced partially from strip chart records. This method, although not highly accurate, provided check points which suggested near normal atmospheric conditions between 81 and 107 km altitude. A computer program is being developed to provide quantitative results for a later publication.

Our preliminary conclusion is that the drag acceleration data show more structure than had been anticipated under the short-term transient conditions of a total eclipse. The results suggest a maximum change in atmospheric structure near totality and a decreasing change near fourth contact.

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Changes in the Electron Content of the Ionosphere

The earliest ionospheric effect of a solar eclipse was noticed by Appleton during the English eclipse of 1927. He detected a four-minute time delay between the minimum in the electron density of the E-region and the time of maximum obscuration. This delay is consistent with the fact that the ionization of the upper atmosphere is caused by ultraviolet radiation rather than by particles emitted from the Sun[1]. Subsequent investigations[2] have revealed that the sources of E-region ionization are somewhat localized over the solar disk, with a significant contribution also emanating from the chromosphere. The rapid response of the E-region to an eclipse is a direct consequence of the short electron recombination times at these heights (~90 s). In the F-region of the ionosphere, however, these recombination times become large (~hours) and the observed eclipse effects are controlled by the process of plasma transport. The chief purpose of the experiment described here was to investigate this transport.

The experiment measured the Faraday rotation introduced by the ionosphere on the 137 MHz transmissions of two geostationary satellites, ATS-I and ATS-III, located respectively at 150° W and 85° W. The geographical location of our observation site at Miahuatlan in Mexico is shown in Fig. 1, which also shows the sub-ionospheric points of various heights along the signal paths to the two satellites. To an observer on the ground, the signal from each satellite is polarized elliptically with a known orientation. The total rotation Ω, in radians, incurred by the satellite signal during its passage through the ionosphere is given by

$$\Omega = 2{\cdot}36 \times 10^4\ \nu^{-2} \int_g^s \{B_0 \cos\theta \sec\chi\} N dh$$

where ν is the wave frequency, B_0 is the geomagnetic field strength, N is the electron density, h is the altitude above the ground and θ and χ are the angles which the signal propagation path makes with the magnetic field and the vertical respectively (mks units are used throughout).

The centre of gravity of the ionospheric electron density distribution is known to be at approximately 350 km. By evaluating H, θ and χ at this height, the measured Faraday rotation can be simply related to the electron contents of vertical columns passing through the sub-ionospheric points A and B shown in Fig. 1. These sub-ionospheric points are at roughly the same latitude (15° N) but are separated approximately by 6° in longitude.

Fig. 2 shows the effective total electron contents at the locations A and B on the eclipse day and on the previous control day. At both locations it is found that, relative to the control day, the electron content starts to decrease after first contact. A minimum in electron content is observed approximately at the time of maximum obscuration. But the maximum reduction of electron content occurs approximately twenty to thirty minutes later than the obscuration maximum. At 102° W (Fig. 2*A*) where the maximum obscuration was 86 per cent, this reduction amounted to $2\cdot3\times10^{17}$ electrons m^{-2}. Somewhat anomalously, the corresponding maximum reduction at 96° W (Fig. 2*B*), where the eclipse was total, was $1\cdot7\times10^{17}$ electrons m^{-2}. These values represent one quarter of the total number of electrons in the ionosphere, and are significantly larger than the reductions measured by Evans[3] and Klobuchar and Whitney[4]. Their measurements were made at high geomagnetic latitude during a period of solar minimum in the month of July.

In order to interpret our observed eclipse effects, it is important to realize that the Faraday rotation technique is primarily sensitive to the F-region and to the topside ionosphere which accounts for approximately 80 per cent of the total content. Because of the large recombination times at these heights, the observed rapid changes in electron content clearly demonstrate the importance of ionization transport. As Evans[3] has noted, such transport is promoted by the rapid decrease of the temperature of electrons (and to a lesser extent of ions) in the topside ionosphere. The transport is essentially aligned with the geomagnetic field. The extent of electron reduction observed in Fig. 2 will therefore depend upon the relative orientation of the geomagnetic field and the lines of sight to each satellite. This geometrical consideration might have some bearing on the anomaly in the maximum electron reductions shown in Fig. 2.

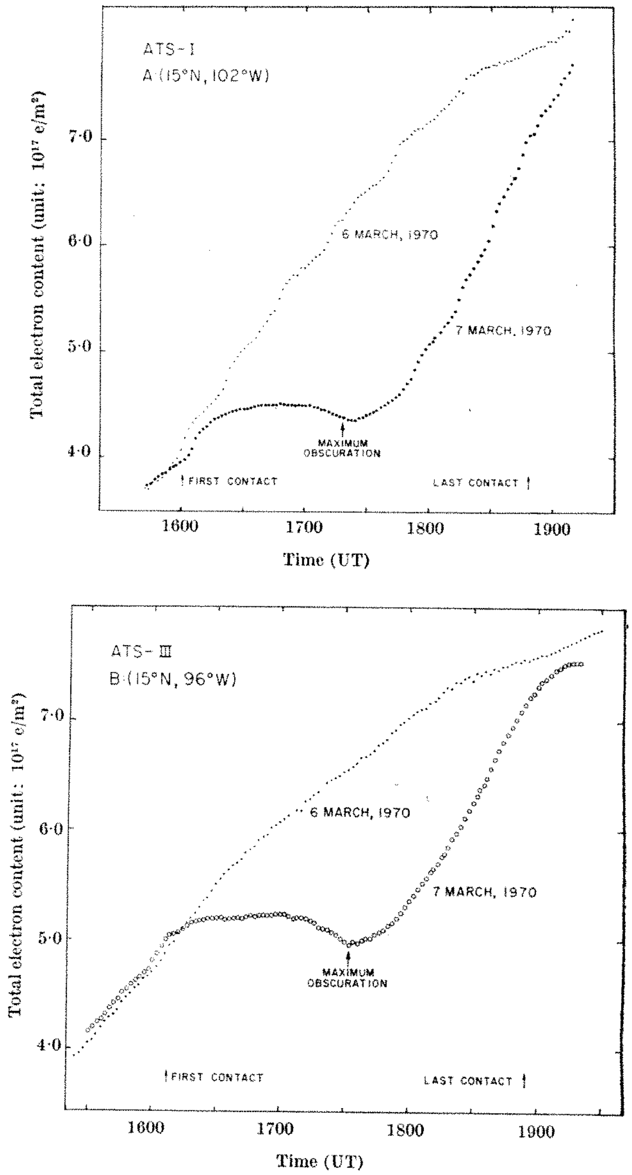

Fig. 2. The effective total electron content at the locations A and B shown in Fig. 1 for the eclipse day, March 7, 1970, and the previous control day.

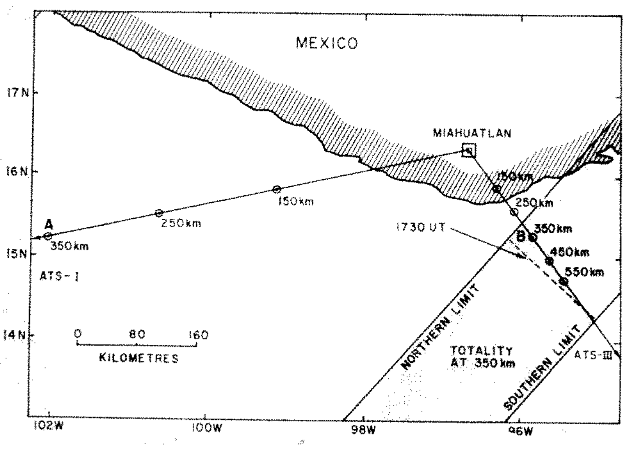

Fig. 1. The location of the observation site showing the path of totality of the eclipse at 350 km, and the lines of sight to the geostationary satellites, ATS-I and ATS-III.

A detailed analysis of our observations is being undertaken using ionosonde data from El Cerrillo near Mexico City. This should enable us to develop a model for transport of ionization in the topside ionosphere.

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Comparison of Changes in Total Electron Content along Three Paths

THE total electron content of the ionosphere during the March 7, 1970, solar eclipse was measured along paths towards three geostationary satellites from a ground station at Hamilton, Massachusetts. The Sun was 96 per cent eclipsed at the ground at Hamilton.

The total electron content (TEC) was determined by monitoring the amount of twist of the polarization of linear, attitude stable, VHF radio waves transmitted from the geostationary satellites Early Bird, ATS-3 and ATS-5. Only the relative polarization twist from each of the three geostationary satellites was monitored, and conversion to absolute polarization twist was as follows, based on measurements of the relative polarization angles at Hamilton, Mass., from the ATS-3 satellite which have been taken on a nearly continuous basis for more than two years. The relative polarization angle is periodically put on an absolute scale by measuring TEC by the two-frequency differential Faraday method from VHF signals transmitted from one of the two S-66 satellites, BE-B and BE-C. The S-66 satellites, BE-B and BE-C, are in approximately circular orbits 1,000 km high having inclinations of 80 degrees and 41 degrees respectively. The S-66 satellites have not been transmitting continuously, and the spacing of their two transmitted frequencies still leaves ambiguities in absolute polarization twist. But it is possible to build up experience in determining absolute TEC from the continuous monitoring of signals from the geostationary satellite over a relatively long period, say at least one month, from calibration by means of S-66 passes which occur before dawn or in late evening, and by comparison with local ionosonde data. An incorrect estimate of the absolute amount of polarization twist will result in unreasonable values of equivalent slab thickness, the ratio of TEC divided by density at the peak of the F-region, particularly at night-time.

By a combination of comparison with BE-C data and f_0F_2 data from the Wallops Island ionosonde, the absolute polarization twist and, hence, the TEC along the path from the satellite ATS-3 was found. The TEC along paths from the satellites ATS-5 and Early Bird were simply scaled to the ATS-3 values at a time before the eclipse.

The amount of polarization twist is proportional to the integrated product of the electron density times the longitudinal component of magnetic field intensity. As is common practice with this type of measurement, the values of polarization twist were converted into equivalent vertical TEC by using the longitudinal magnetic field intensity and zenith angle at a mean ionospheric height—350 km in this case. The resultant equivalent vertical TEC values were then assigned to the subpoints 350 km ionospheric height. Because the longitudinal component of the magnetic field times the zenith angle changes slowly as a function of height, an error in TEC of less than 8 per cent would have been introduced along each path by choosing an unlikely high height of 500 km rather than 350 km.

Fig. 1 shows the location of the receiving station and the 350 km sub-ionospheric points along the paths to the three satellites. Also shown are the locations of the ionosondes which were operating during the eclipse, Fort Monmouth, New Jersey, and Wallops Island, Virginia, and the ground projection of the sub-ionospheric path of the eclipse shadow at a height of 300 km. The eclipse shadow at 300 km was approximately 130 km wide[1].

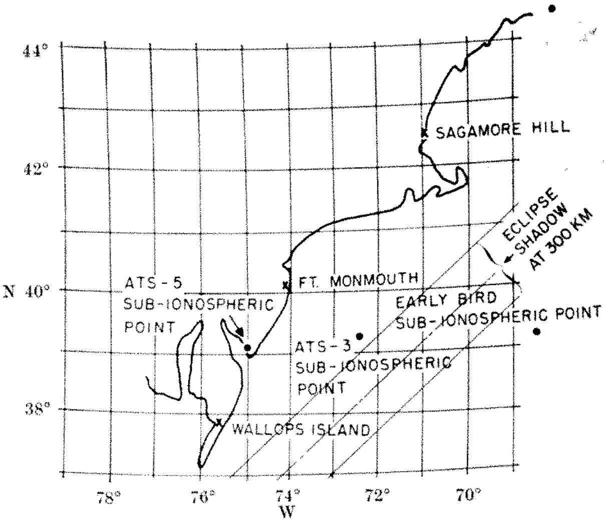

Fig. 1. Map of the area.

Fig. 2 shows the equivalent vertical TEC results during the eclipse day, along with the mean of the three days preceding the eclipse. Early Bird data were not available early on the eclipse day because the VHF transmitter was turned off for a satellite manoeuvre. The three curves for the eclipse day are nearly identical, with the exception that the recovery along the Early Bird path was not as great as along the other two paths. The path from the Early Bird satellite was furthest east, or latest in local time, and could be expected to recover least of the three paths because of the lower solar elevation angles along its path.

The maximum change in TEC from the mean of the three preceding days was approximately −30 per cent. The time delay from the time of maximum optical obscuration to the observed minima in TEC along the three paths was approximately 20 minutes. No significant differences in time delay were noted along the three paths.

Vertical incidence ionosondes located at Wallops Island and Fort Monmouth observed decreases of density at the peak of the F-region of approximately 30 per cent. The

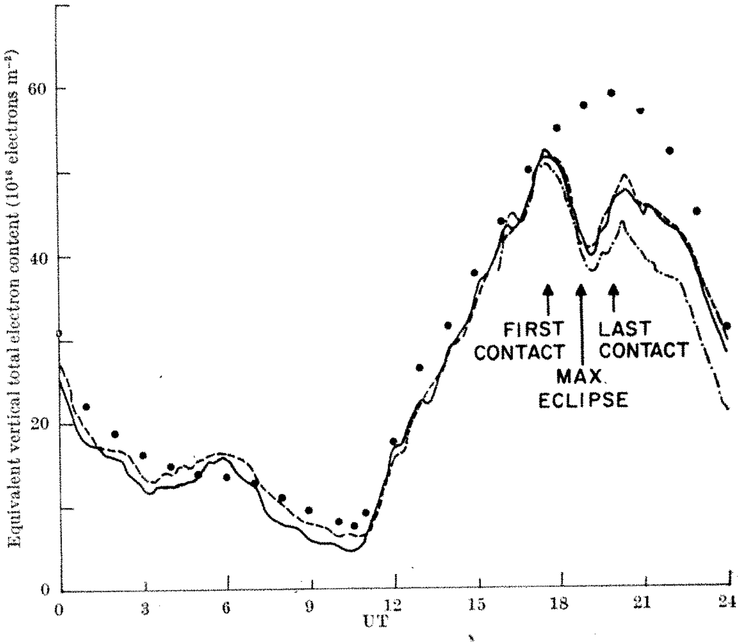

Fig. 2. Equivalent vertical total electron content on the eclipse day and on the three days preceding the eclipse. ———, ATS-3; . . . , smoothed mean of March 4, 5 and 6; - - -, ATS-5; —·—·—, Early Bird.

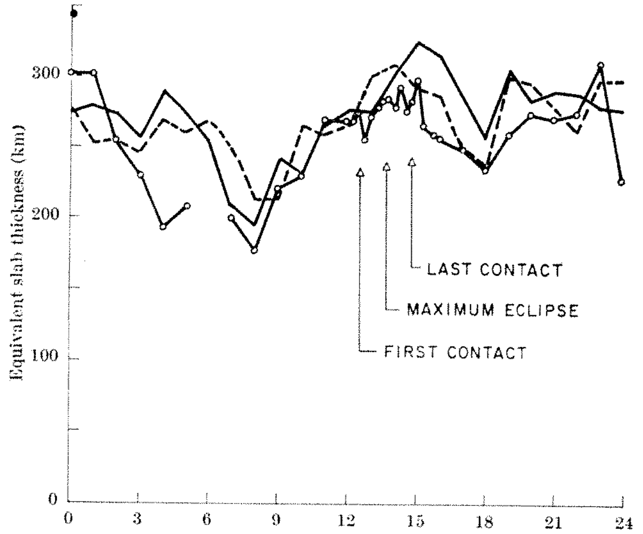

Fig. 3. Equivalent slab thickness of the F-region. ———, March 5; - - -, March 6; ○—○, March 7.

equivalent slab thickness of the F-region, shown in Fig. 3, was not significantly changed during the eclipse, at least not when compared with the two days preceding the eclipse. All slab thickness values shown in Fig. 3 were computed using TEC values from the ATS-3 path and ionosonde data from Wallops Island.

In spite of the fact that the solar eclipse occurred during a moderately disturbed period ($\sum_{24} K_{\mathrm{Fr}} = 33$) the eclipse effects were clearly predominant, in contrast to a report by King *et al.*[2], who observed that storm effects predominated during the June 10, 1964, eclipse near Woomera, Australia. The March 7, 1970, eclipse was also interesting in that TEC and $N_{\max}$ changed by approximately the same amount. This indicated that the expected piling up of electrons from higher heights to near the peak was more than compensated for by losses at the peak such that layer shape was maintained.

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Synoptic Preview of Ionospheric Data taken at Fort Monmouth, New Jersey, during the Eclipse

Various ionospheric observations were made at Fort Monmouth during the eclipse of March 7. These included a readout from the topside sounder satellite Alouette 1, polarization and signal strength measurements from beacons in the S-66 and ATS-3 satellites, and bottomside ionograms from various locations. This letter is a preliminary view of how data from the ATS-3 observations are related to the vertical ionogram data obtained at Wallops Island kindly provided by R. S. Gray, ESSA Ionospheric Station, Wallops Island. We selected the Wallops Island ionograms for our comparison because the sub-ionospheric point (below 350 km altitude) for our path to ATS-3 is at 37·1° N, 75·5° W, rather close to the Wallops Island station at 37·9° N, 75·5° W.

The polarization measurements of the ATS-3 beacon (137·35 MHz) represent the expected picture for a solar eclipse close to local noon. Before first contact, the polarization angle (the total electron content) increased steadily. Then a turning point (or maximum) was reached when the data decreased to a minimum related to the maximum coverage of the eclipse. After the minimum the polarization increased until a second turning point (maximum) was observed. From then on the polarization descreased as it would during the afternoon of an undisturbed day. A similar variation was observed for the plasma frequencies, for example, F_0 and E_0, read from the ionograms.

At an altitude of about 300 km, first contact at the sub-ionospheric point was at 1225 EST. The first turning point in the ATS-3 observations, that is, the moment when the effects of decreasing ionization is noticed in the total electron content, was observed at 12(38 ± 3) EST, about 13 min after first contact. The ionograms from Wallops Island show the beginning of a decrease of the plasma frequencies in the F-region at 12(45 ± 4) EST. This time delay is more than can be explained by the more northern position of the ionosonde in comparison with the sub-ionospheric point—we estimate a delay of about 1–2 min due to the difference between locations. Thus we conclude that the effect of the eclipse is revealed in the total content along the path of the satellite about 4 min earlier than in the F-region observations. The E-region data from Wallops Island show the first reaction at 12(15 ± 2) EST, earlier than the time for first contact as seen from the ground at Wallops Island (1220 EST), and also earlier than the first contact time at 300 km altitude for the sub-ionospheric point (1225 EST). Although more detailed investigations are necessary, the early decrease of E_0 before the beginning of the decrease in total content seems to be compatible with the later reaction of the F-region.

Maximum coverage at the sub-ionospheric point (at 300 km) is estimated as 1343 EST. At this time the polarization data from ATS-3 indicated a reduction of only about 90 per cent of the total reduction observed. The minimum was reached at 14(06 ± 3) EST, 23 min after maximum coverage. The F-region minimum at Wallops Island was at 14(15 ± 8) EST. Again assuming a two min delay because of the distance between the two locations, there is a delay between the minimum in total content and the F_0 minimum of about 7 min. Again the E-region responds more quickly, reaching minimum at 1340 EST at Wallops Island (equivalent to 1338 EST at the sub-ionospheric point). This appears to be earlier than the time of maximum coverage at the E-region altitude.

The total content data resumed their normal decrease at 15(26 ± 3) EST. The F-region data reached normal values at 15(30 ± 8) EST and the E-region at 1500 EST. Last contact at the sub-ionospheric point was about 1458 EST. Again assuming the time difference of 2 min between locations, we find the E-region time coinciding with full illumination. F-region recovery is delayed by 30 min and recovery of total content by 28 min.

The data are consistent in so far as the comparison of F-region data with total electron content data is concerned, but the reaction of the E-region seems to be advanced in time relative to changes in illumination. This might have something to do with the distribution of zones of X-ray activity (Bomke *et al.* at the spring meeting of the US National Committee of URSI, 1970).

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Observations of Ionospheric Electron Content during the March 7, 1970, Solar Eclipse

FARADAY rotation measurements of the VHF transmissions from the geostationary satellites ATS-I and ATS-III were carried out at several of our observatories in the United States during the eclipse. In addition, at Eastville, Virginia (37° 20′ 50″ N, 75° 54′ 13″ W), near Wallops Island, we measured the changes in the difference of the VHF and UHF phase path length between the ground and ATS-III. This satellite had been moved to a longitude of 85° W to permit a relatively good alignment of the geomagnetic field with the ray path from ATS-III to Eastville. This path crossed an eclipsed part of the F-region.

Fig. 1 shows the curve of the electron content versus time for Eastville on March 7, 1970. Corresponding curves obtained at Greenbelt, Maryland, and Rosman, North Carolina, show very similar behaviour. All these sites observed a volume of the F-region in which the eclipse was above 95 per cent. At Eastville the bulk of the ionosphere was totally eclipsed. In Fig. 1 a pronounced bite-out is seen which, using the previous day as a control day, can be estimated as about 20×10^{16} electrons m^{-2}. The relative bite-out was ~30 per cent. A 35 minute delay was observed between the time of minimum electron content and the time of totality.

The slab thickness, τ, defined as the ratio between electron content and electron concentration at the F2 peak, behaved in an interesting fashion. Some minutes prior to first contact it had a value of 430 km, which persisted up to 30 min before the maximum of totality when it fell rapidly, reaching a minimum value of 422 km at the middle of totality, probably due to the wiping out of the E layer. It then rose sharply to 480 km at the time the electron content reached its minimum value and decayed again to some 400 km after the fourth contact. In the time interval between totality and fourth contact τ took a sharp dip to 440 km, only to recover, reaching again 470 km.

Because the cooling of the upper ionosphere expected during the eclipse would reduce τ, the observed increase may be due to a marked erosion of the O^+ layer and consequent lowering of the $O^+ - H^+$ transition height.

The bite-out in electron content at stations away from the path of totality was surprisingly low, as indicated in Table 1.

Table 1. SIZE OF THE BITE-OUT IN ELECTRON CONTENT

	Eclipse magnitude (per cent)	Bite-out (per cent)
Eastville	≈100	≈33
Fort Collins	45	non-detectable
Clark Lake	28	non-detectable
Stanford	26	non-detectable

From the measurements at Eastville, both the columnar electron content I and its time derivative $\dot{I}$ were calculated. The latter is determined with great accuracy from the phase path length experiment.

As expected, near the end of totality—1836.30 UT—the rate of change of the columnar content reached its largest negative value of $(-7\cdot79 \pm 0\cdot13) \times 10^{13}$ electrons m^{-2} s^{-1}. This value can be introduced into the continuity equation to yield a value of the linear loss rate $\langle\beta\rangle$:

$$\langle\beta\rangle = \frac{-\dot{I} + \varphi + Q}{I}$$

where $\langle\beta\rangle$ is the weighted mean of the loss rate, φ is the excess inflow over outflow of plasma in the column of unit base between observer and satellite and Q is the total electron production rate in the column. Both φ and Q are unknown quantities and their upper limits must be estimated.

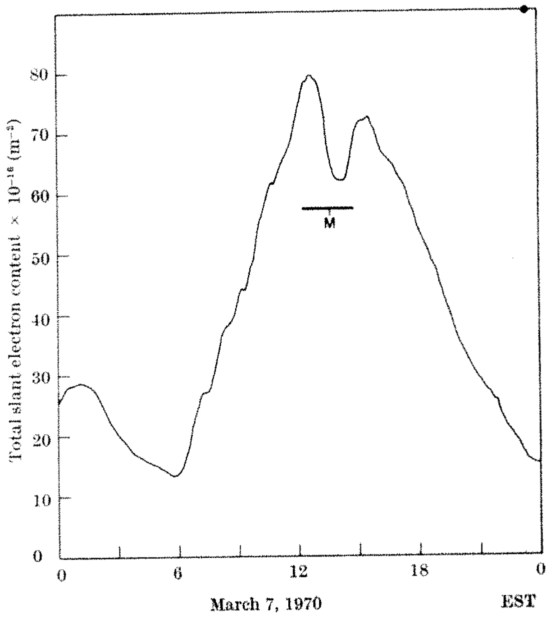

Fig. 1. Slant columnar electron content along the ATS-III to Eastville, Virginia, path. The values of electron content were determined by the method of Almeida *et al.*[3]. The horizontal bar indicates the duration of the eclipse.

The integrated production rate for an overhead Sun[1] was about 5×10^{14} electrons m^{-2} s^{-1} in 1966 when the solar 2,800 MHz radio flux was 100×10^{-22} W m^{-2} s^{-1} Hz^{-1}. If there is proportionality between radio flux and production rate, the overhead Sun production rate was about 8×10^{14} electrons m^{-2} s^{-1}, assuming a solar flux of 150×10^{-22} W m^{-2} s^{-1} Hz^{-1}. The actual production rate corrected for the solar zenith angle of 47° at Eastville at 1836 UT would be 5×10^{14} electrons m^{-2} s^{-1} if the eclipse had not occurred. At totality most of the light output from the Sun vanishes, but at X-ray wavelengths substantial radiation persists[2]. It is not known to what extent the EUV radiation responsible for most of the F-region ionization is obscured by the Moon, but if 99 per cent of the significant radiation is removed by the eclipse, then Q falls to a value of one order of magnitude lower than $\dot{I}$.

To assess the contribution of the Φ term, consider the plasma flux as divided into two components, one along the ray path and one normal to it. The parallel component has a vanishing integral along the column because the flux is certainly zero at the lower limit of integration near the observer and it is very nearly zero in the upper limit at the satellite not only in view of the small electron concentration (the spacecraft is beyond the plasmapause) but also because the geomagnetic field lines make a steep angle with the ray path to the satellite. Thus the only contribution to Φ is due to the normal component of flux which even using a very generous estimate is two orders of magnitude smaller than $\dot{I}$, so that the flux term has little influence in the determination of the value of the recombination coefficient.

The errors in $\langle\beta\rangle$ are therefore due to errors in measuring I and $\dot{I}$ and uncertainties in the estimation of Q during totality. If one disregards this latter influence, one obtains

$$\langle\beta\rangle \approx \frac{-\dot{I}}{I} = \frac{7\cdot79 \pm 0\cdot13}{(66\cdot8 \pm 2\cdot0)}\, 10^{-3} = (1\cdot16 \pm 0\cdot05)\, 10^{-4}\ s^{-1}$$

The determination of $\langle\beta\rangle$ from Faraday rotation measurements meets several difficulties not found with the phase path length technique. One obstacle is in the difficulty of measuring the time rate of change of the Faraday rotation angle Ω. This determination is quite "noisy" compared with the very high quality of the phase path change data. In addition, ground reflexions do not perturb the latter measurement but significantly alter the value of Ω and, because these reflexions are themselves affected by the polarization angle of the received wave, they have a marked influence on $\dot{\Omega}$.

An additional source of error comes from the fact that Ω changes not only in response to variations in electron content but also in response to alterations in the shape and the height of the layer. We have

$$I = \Omega / G$$

$$\langle \beta \rangle \approx \frac{-\dot{I}}{I} = -\frac{\dot{\Omega}}{\Omega} + \frac{\dot{G}}{G}$$

Because from the measurement of the Faraday rotation alone G and $\dot{G}$ cannot be estimated[3], the second term in the equation may introduce an appreciable error in ⟨β⟩. By combining phase path length and Faraday rotation measurements, both G and $\dot{G}$ can be estimated[3]. This was done at Eastville with the following result (at 1836.30 UT)

$$G = 5{\cdot}0 \times 10^{-17} \text{ rad m}^2$$

$$\dot{G} \cong -5{\cdot}5 \times 10^{-21} \text{ rad m}^2 \text{ s}^{-1}$$

Thus $$\frac{\dot{G}}{G} = -1{\cdot}1 \times 10^{-4} \text{ s}^{-1}$$

a value comparable with the value of ⟨β⟩. The use of Faraday rotation angle alone for the determination of the mean recombination coefficient may therefore lead to errors of the order of 100 per cent.

At present electron concentration profiles from the Wallops Island ionosonde are not yet available. When these data are available it is hoped that by investigating the behaviour of τ, G, and $h_{\max}$ during the eclipse, good information about the layer changes induced by the eclipse can be derived.

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Variation of Electron Density in the D-region

This letter reports some tentative results of measurements of the D-region electron density made at the Pennsylvania State University during the solar eclipse of March 7, 1970, by the wave interaction technique described by Lee and Ferraro[1].

At Pennsylvania State University the eclipse was partial with about 90 per cent obscuration of the solar disk at maximum, which occurred at about 1838 UT. No data were obtained before 1809 UT because of a power failure and hence conditions before the eclipse are not known. The time required to obtain a profile was about seven minutes. This time could have been shortened to about two minutes, but as the interaction effect was expected to decrease during the eclipse the long integration time was adopted to improve the signal to noise ratio. In addition, rapid changes in electron density were not expected because the eclipse was only partial. An experiment to measure the electron energy loss coefficient, requiring about twenty minutes, was performed after every third electron density measurement; as a result, electron density measurements were not made continuously. The results of the energy loss coefficient experiment are described on page 1119.

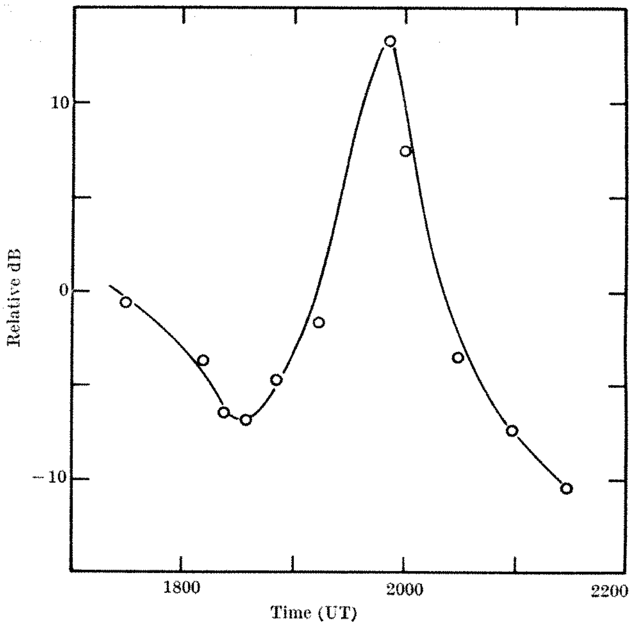

Fig. 1. Vertical incidence absorption measured at 2·2 MHz, March 7, 1970.

Fig. 1 shows the relative values of the measured vertical incidence absorption at 2·2 MHz. This information was obtained continuously and then averaged over intervals of seven minutes. The main eclipse effect at about 1830 UT and the effect of some other geomagnetic disturbance at about 2000 UT are clearly seen. Information on the effects of the X-ray flare which occurred at about 1610 UT was lost because of the power failure.

Fig. 2 shows electron density profiles for 1809, 1828, and 1913 UT on March 7, 1970, and for 1810 UT on March 11, 1970, for comparison with a normal day. These times

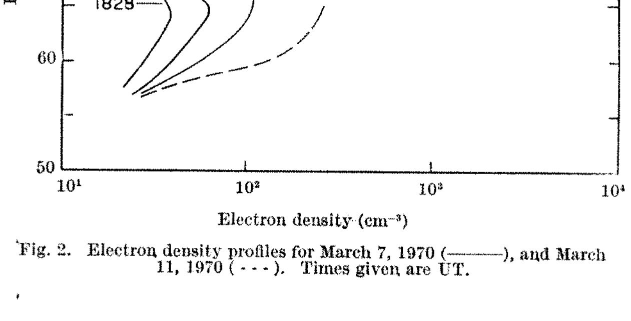

Fig. 2. Electron density profiles for March 7, 1970 (———), and March 11, 1970 (- - -). Times given are UT.

refer to the centre of the measurement interval of seven minutes. The chief eclipse effect seems to be above 70 km, with the electron density at lower heights remaining approximately constant until the event after the eclipse.

The interpretation of these results in terms of D-region chemistry is affected by at least two factors, the absence of pre-eclipse data at this location and the fact that the Sun was active during the period of the eclipse. The latter fact may be beneficial in that the covering and uncovering of active areas of the Sun may cause the 1–8 Å X-ray emission and the Lyman-α emission to vary differently during the eclipse and allow their effects to be sorted out. The absence of pre-eclipse and pre-flare data is a more serious problem as the response of the ionosphere to the eclipse will depend to some extent on how much it had recovered from the effect of the flare. Rowe *et al.*[2] have presented an analysis of the changes in electron density during a solar flare, which allows limits to be placed on the nitric oxide concentration. This analysis involves integration of the electron continuity equation starting at a point where the ionosphere is in equilibrium. In the present case such a starting point is not available; the analysis may have to be performed backwards, starting from a final point.

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Steep-Incidence D-region Reflexions of Low Frequency Pulses during the March 7 Eclipse

THE path of totality of the eclipse of March 7, 1970, passed very near the Loran-C transmitter at Cape Fear, North Carolina. Loran-C transmissions are pulses with a carrier frequency of 100 kHz, a nominal bandwidth of 20 kHz, a nominal peak radiated power of 400 kW and are vertically polarized. The complete pulses of the ground-wave and the once reflected sky-wave signals were recorded by synchronized averaging techniques at a site 48·87 km west of the transmitter (azimuth 258·3°). The signals from a loop antenna in the plane of incidence (normal loop), a loop antenna perpendicular to the plane of incidence (abnormal loop), and a short whip antenna were recorded on separate channels at the receiving site. The data were later spectrum analysed, and the normal loop and abnormal loop measurements were used to derive the normally reflected and abnormally reflected sky-wave amplitudes at several frequencies in the Loran-C frequency band. The sky-wave amplitudes are normalized to the ground-wave signal as measured at the receiving site using the normal loop. Ionspheric reflexion coefficients can be deduced from these data after allowance for antenna radiation patterns and reciprocal distance effects.

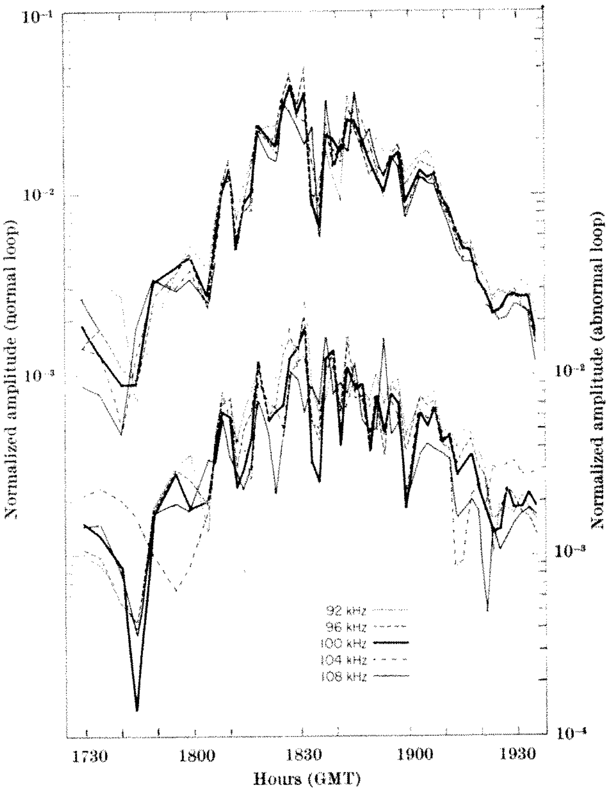

Fig. 1. Normalized sky-wave amplitudes for the normal loop (upper curves and left hand scale) and the abnormal loop (lower curves and right hand scale) as a function of time. Totality occurred at 1831 GMT.

Fig. 1 shows the magnitude of the normalized sky-wave amplitudes for five frequencies as a function of time during the eclipse. At D-region heights the first contact occurred at about 1711 GMT, the centre of totality at 1831 GMT, and the fourth contact at about 1948 GMT. Because of a certain amount of weak ground clutter in the signals, the sky-wave amplitudes both at the start and near the end of the eclipse, based as they are on weak ionospheric reflexions, are of doubtful significance. The measurements from about 1810 GMT to about 1920 GMT contain negligible spurious signals for two reasons: (1) the wanted signal-to-clutter ratio was large, and (2) the pulse delays were great enough during the main phase of the eclipse for the ground clutter to be separated out temporarily. It is these data that we would like to discuss. At the lowest and highest frequencies for which data are given, the transmitted signal amplitude is within the half amplitude points of the spectral distribution.

Both the normal loop antenna and the whip antenna respond to the normally reflected sky-wave signals. Because of differences in the antenna radiation pattern, the angle-of-arrival of the sky wave can be deduced. Fig. 2 shows the angle of arrival thus deduced (measured from the vertical) for the five frequencies used in Fig. 1. For our geometry, the angle of arrival is nearly equal to the angle of incidence on the ionosphere.

The most striking feature of these data is the oscillatory characteristics of the normalized sky-wave amplitudes. These characteristics also appear regularly during the sunrise and sunset transition periods on normal days. We believe the oscillatory feature results from interference between signals reflected from different levels in the D-region. The lower levels must, of course, be only partially reflecting. As the electron density distribution in the D-region changes during the eclipse, these reflecting levels can move with respect to each other, producing a pattern of alternating periods of constructive and destructive interference superimposed upon the general trends. In a smoothly varying ionosphere, levels of possible partial reflection are at heights $X = 1$, a region of strong coupling between ordinary and extraordinary waves, and at $X = (1 - Y^2)/(1 - Y_z^2)$, a level at which the equations describing the propagation have a singularity in the absence of collisions[1]. In a stratified ionosphere, likely contributors to partial reflexions are those levels in the D-region where a layer of enhanced (or diminished) ionization exists within what would otherwise be a monotonically increasing ionosphere. That such layers occur is indicated by the angle-of-incidence data. Periodically (for example, at 1814, 1823, 1835, and so on), the high frequencies show angles of arrival considerably smaller than the low frequencies. This can be interpreted in terms of a two-layer D-region; the low frequencies are reflected by a lower layer, while the high frequencies penetrate the lower layer and are reflected by the upper layer.

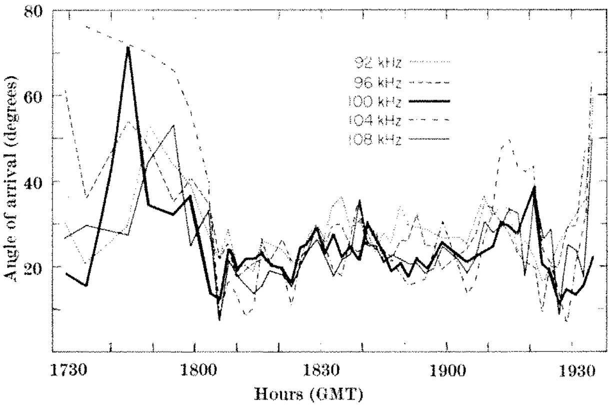

Fig. 2. Angle of arrival (measured from the vertical) as a function of time. The frequencies are the same as those used in Fig. 1. The data show that the various frequency components in the pulse appear to come from different parts of the sky.

The angle-of-arrival results (Fig. 2) are unexpected. They demonstrate that the different frequencies comprising the pulse appear to arrive from different parts of the sky. The most surprising aspect of the data is that at times the difference in angle of arrival of the various frequencies is as much as 20°, and that some of the angles of arrival correspond to surprisingly low "triangulation heights" of reflexion. Based on stationary phase concepts, a "triangulation height" of reflexion can be shown to be proportional to $\partial\psi/\partial I$, the rate of change of phase of the reflexion coefficient with angle of incidence[2]. Model profiles of electron density used in preliminary calculations for steep-incidence reflexion at these frequencies have yielded results showing that under certain circumstances $\partial\psi/\partial I$ can be very small indeed; hence the height of reflexion may appear to be lower than the height where the actual reflexion process occurs.

We have assumed that the D-region can be considered to be horizontally stratified. For a tilted, or rough region, the data would be very difficult to interpret. The response of the several frequencies in the Loran-C band that we have examined seem to be sufficiently uncorrelated that we have, in effect, a "broad-band" multifrequency sounding of the D-region. We believe that the data, despite their complexity, can be interpreted in terms of a horizontally stratified D-region and, within the framework of this model, it is our objective to derive from these data as much detailed information as possible about D-region structure.

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Preliminary Results of a Radio Absorption Study at 3·5 MHz

The D-region of the ionosphere (50 to 90 km) is the main absorbing layer for radio waves. This is because in this relatively dense region the collision frequency is of the same order of magnitude or greater than the frequency of the absorbed waves. Electrons excited by a wave tend to scatter away the energy as heat rather than reradiate it as a wave. To a lesser extent the same is true of the E-region (90 to 150 km). Thus any phenomenon which depletes the D-region ionization, such as a solar eclipse, will decrease absorption. On the other hand, any phenomenon which increases the ionization, such as a solar flare, will produce an increase in absorption. These effects, therefore, can shed light on D-region processes. A continuous wave (A3) experiment at 3·57 MHz was conducted during the March 7 eclipse to study the influence of the eclipse on the absorption.

One interesting aspect is that the eclipse occurred at a time of intense solar activity. The days about the eclipse were, to varying degrees, magnetically disturbed, and several solar flares occurred on March 6, 7 and 8. The west limb flare on March 6 was responsible for a very intense magnetic storm and aurora on March 8.

King, Legg and Reed[1] have found that disturbed magnetic conditions can obscure completely at least some eclipse effects. The bottomside measures of f_0F2 displayed no eclipse effects during the disturbed eclipse of June 10, 1964, when the magnetic index, K_p, reached a maximum of 7. On March 7, 1970, however, magnetic conditions, though disturbed, were considerably quieter than this with K_p reaching only 4_+.

A 75 W transmitting station was set up at Waycross, Georgia, near the centre of the region of totality, and the receiving station was at Steinhatchee, Florida, some 190 km to the south-west and also along the path of totality. Each used half-wave dipoles, orientated parallel to the eclipse track to prevent any possibility of contamination of the desired sky-wave signal with ground waves. As the sky-wave for this base line and E or F layer reflexion heights must depart and arrive at angles of less than 45° to the vertical, the sky-wave is not attenuated appreciably by this configuration.

On the eclipse day, the signal level was sampled approximately every 10 min except for the period near totality when samples were every 1 to 2 min. Each sample was the mean of ten consecutive pulses, each 2 s

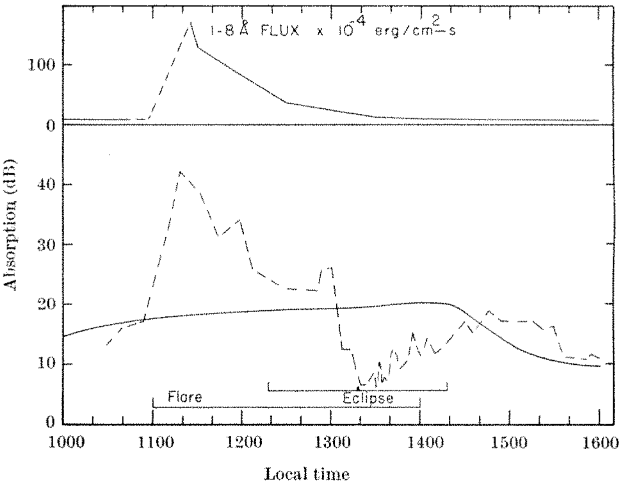

Fig. 1. Ionospheric absorption. In the lower plot, the broken line represents the observed absorption on the day of the eclipse, and the solid line represents the mean of the absorption on the control days. The upper plot is the 1–8 Å X-ray flux detected by the Solrad satellite. The broken lines represent a period at the beginning of the flare when the satellite was eclipsed by the Earth.

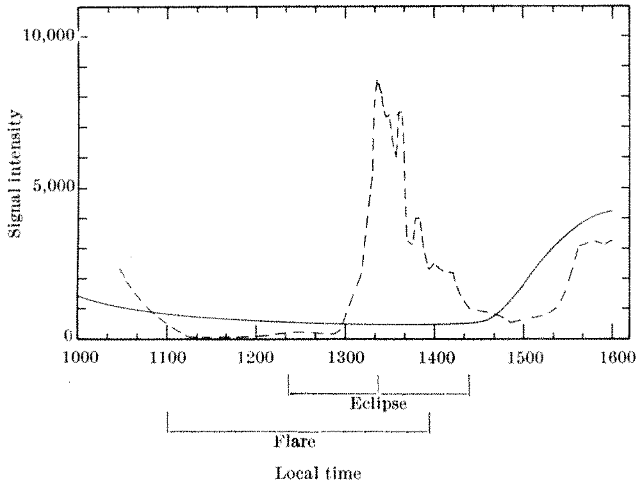

Fig. 2. Received signal intensity. The broken line represents the smoothed intensity of the received signal (the reverse of absorption) plotted on an arbitrary linear scale. The solid line represents the mean of the intensities on the control days.

long, emitted by the transmitter. On the control days, March 8, 14 and 15, the signal level was sampled at intervals of either 5 or 10 min.

Fig. 1 shows the relative absorption measured on March 7 as compared with the mean for the control days. For this preliminary report the zero level has been established arbitrarily at the theoretical value confirmed by Appleton and Piggott[2]. A solar flare of importance-2 occurred on the morning of the eclipse, about 2 h before the passage of totality. Consequently, Fig. 1 shows not only the anticipated decrease in absorption produced by the eclipse, but also a significant increase in absorption induced by flare radiation prior to the eclipse.

Fig. 1 also shows the evolution of the solar flare as seen in the 1–8 Å X-ray band from the Solrad satellite. There is a strong correlation between the X-ray flux and the observed absorption. The eclipse occurred near a sunspot maximum when X-rays in the 1–8 Å band are probably the chief source of D-layer ionization, although Lyman-α contributes to some degree[3].

After an approximate correction for the effect of the flare, we find that the maximum change in absorption produced by the eclipse is of the order of 14 to 15 dB. This is in good agreement with that obtained by the pulse sounding (A1) measurements at 3·86 MHz conducted by Bischoff and Taubenheim[4] during the annular eclipse of May 20, 1966. They also found an anomalous decrease in the absorption beginning some 28 min after the minimum absorption had been reached. Although somewhat obscured by the scatter in Fig. 1, in Fig. 2 (where the data have been smoothed and plotted on an intensity scale) a similar anomaly is seen to occur about 26 min after the absorption minimum.

From Fig. 2 it can be seen that the intensity of the received signal increased sharply between 1300 and 1320, remained relatively high for some 20 min, and then decreased sharply by a factor of 2·5 at 1340. With the anomalous rise in signal at 1346, the slope of the decay takes on a much more gradual appearance. Fig. 2 also shows that the centre of the sharply peaked region occurs at about 1328, some 8 min later than the time when the centre of the umbra passed the path midpoint at 80 km.

Finally, it seems that the decrease in absorption which consistently occurred on the control days by 1440 at the latest, as the transition to night-time conditions began, did not occur on March 7 until at least 1520. This 40 min delay seems to be real, but at present it is not certain whether it is the result of the flare, the eclipse or some other influence.

We thank Drs S. S. Prasad and T. D. Carr for suggesting the experiment and for valuable criticism, and Messrs Donald Roberts, Jerry Allnoch, Reavis Dixon and John Bridges for help.

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Electron Energy Loss Factor in the D-region during the Eclipse of March 7, 1970

THIS letter describes the experimental observation of the electron energy loss factor (G) in the D-region during the eclipse. The experiment described is still in the development stage, but seasonal variations in G have been seen.

The G factor is important for the reduction of interaction experiments at the Ionosphere Research Laboratory, especially to describe experiments on the Luxembourg effect. The G factor enters the theory by way of the electron energy balance equation, which is used to obtain the excess electron energy (above ambient) that is imparted by a radio wave. This equation as given by Ratcliffe and Shaw[1] is

$$\frac{d}{dt}(\Delta Q) + G\nu\,(\Delta Q) = P(t)$$

where ΔQ is the excess electron energy, ν is the collision frequency, and $P(t)$ is the mean power supplied to an electron by the wave. G then represents the mean excess energy lost by an electron per collision.

The experiment is a variation of a disturbing-before-wanted (DBW) experiment used by Smith *et al.* (unpublished). Another form of interaction experiment is used at the Ionosphere Research Laboratory to determine the electron density profile (N) and the collision frequency profile (ν) (see, for example, ref. 2). In the DBW experiment the disturbing pulse is transmitted before the wanted pulse. The amplitude and phase interaction coefficients are then plotted as a function of the time spacing between the two pulses. Using a catalogue of disturbing-before-wanted simulation, G can be determined without the knowledge of the electron density collision frequency, with some range of error, because the DBW experiment is relatively sensitive to G. The use of both the extraordinary (X) and ordinary (O) mode disturbing wave gives a method of obtaining G with more reliability than is possible with either mode alone.

As mentioned earlier, seasonable variations in G have been seen showing a decrease in the winter. The average amplitude and phase interaction coefficients for June 1969 compared with those of January 1970 are shown in Fig. 1. The slopes of these curves are all that is meaningful for the determination of G; therefore the absolute levels are unimportant. The slope depends on N and ν somewhat, but simulations have shown their effect to be small compared to the effect of G. Physically, this is because at larger time delays the electron energy is lower with a larger G due to greater loss at each collision. The plots shown in Fig. 1 were compared to the DBW simulation catalogue. During June 1969, G was between $3{\cdot}5 \times 10^{-3}$ and 5×10^{-3}, and during January 1970, between $2{\cdot}0 \times 10^{-3}$ and $3{\cdot}5 \times 10^{-3}$.

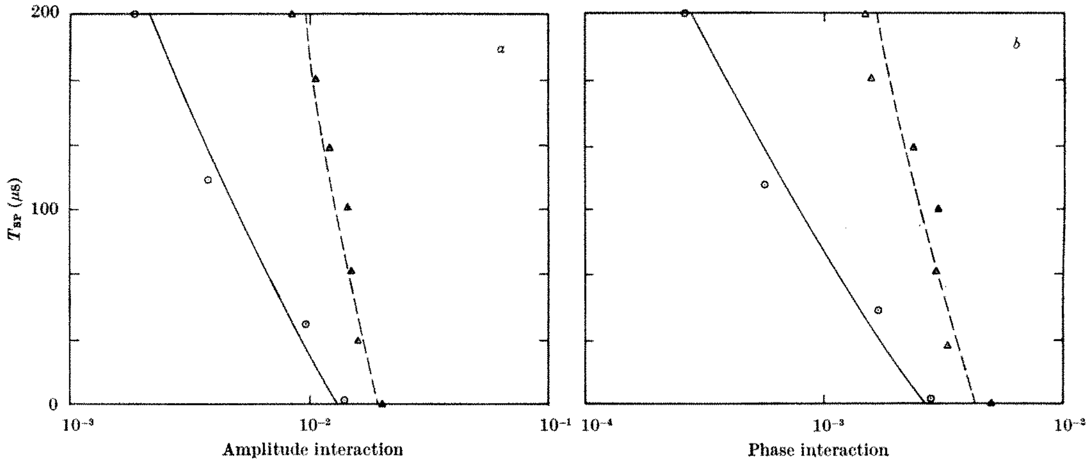

Fig. 1. Amplitude interaction (*a*) and phase interaction (*b*) for June 1969 (○—○) and January 1970 (△ - - - △), using X mode disturbing.

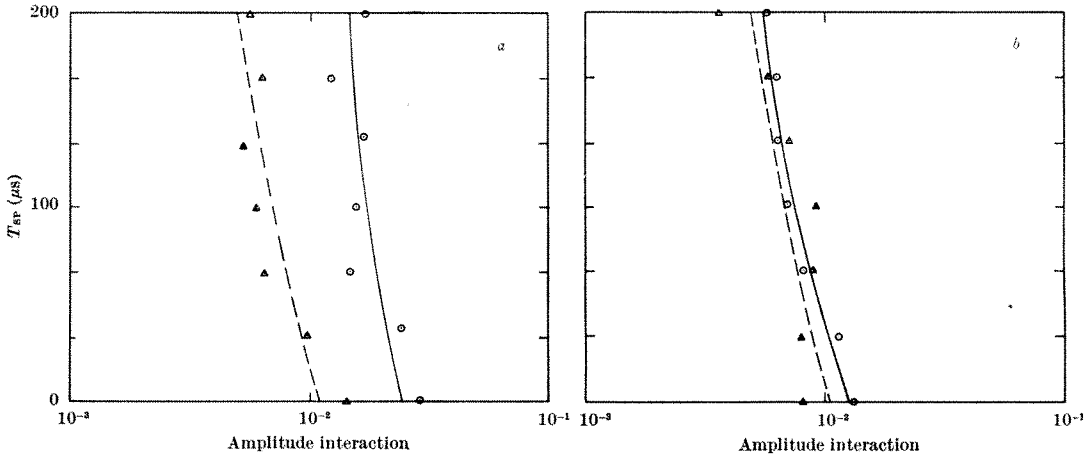

Fig. 2. Amplitude interaction for 1834 GMT, March 7, 1970 (△ - - - △), using (*a*) X mode disturbing and (*b*) O mode disturbing. ○—○, Reference.

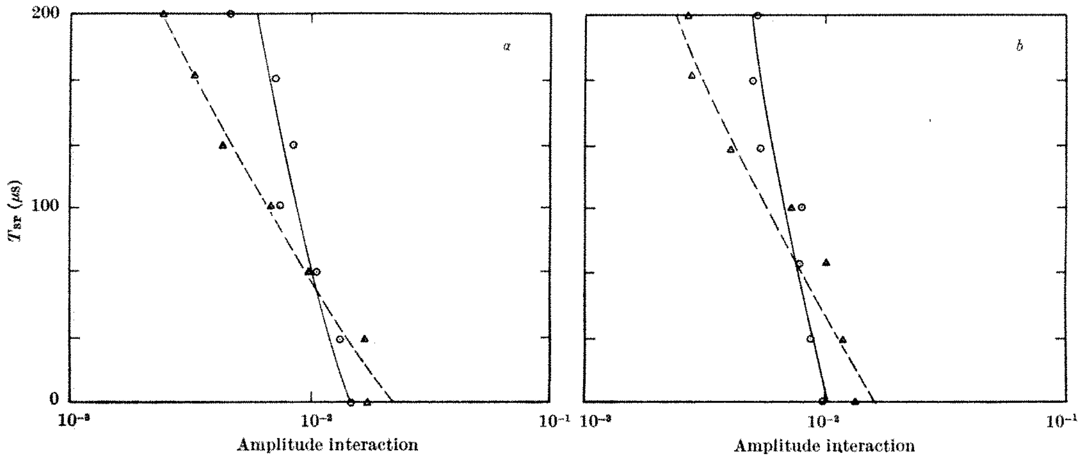

Fig. 3. Amplitude interaction for 1955 GMT, March 7, 1970 (△ - - - △), using (*a*) X mode disturbing and (*b*) O mode disturbing. ○—○, Reference.

Dalgarno and Moffett[3] calculated the theoretical temperature dependence of *G* in air to be approximately $T^{-3/2}$. Huxley[4], in a more detailed derivation, found this dependence to be $T^{-5/2}$ for air.

The experiment was run during the eclipse to search for a possible drop in the D-region temperature, which would show up as a rise in *G*.

The results will be shown in the form of a comparison of interaction coefficients for March 7 and an average of interaction coefficients for several days before and after the eclipse. This is done for different times during the eclipse, and each run was compared to those taken at about the same time on the reference. The reference used is an average of DBW interaction coefficients for February 25 and 26 and for March 10 and 11, 1970. Unfortunately, due to a power failure, no data just prior to the eclipse are available. It is also unfortunate that the phase interactions for March 7 are too noisy to use.

In Fig. 2*a* and *b* the curves for March 7 at 1834 GMT are compared with the reference, for the X and O mode disturbing respectively. The slopes compare well, so we conclude that there was no noticeable *G* change at 1834

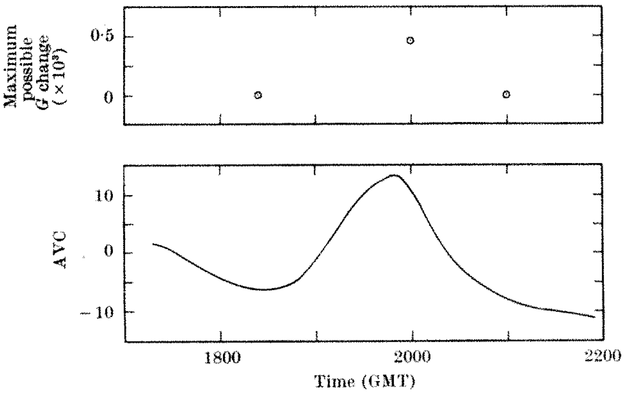

Fig. 4. Comparison of possible G change and receiver AVC.

GMT due to the eclipse. Fig. 3 shows the same curves for 1955 GMT. The March 7 data show a smaller slope than the reference implying a rise in G. Because there were no phase data, the change in G that this slope change represents is more uncertain. This change in G is estimated to be about $0\cdot2\times10^{-3}$ to $0\cdot5\times10^{-3}$ taking into account preliminary N studies made at the laboratory for the same times. Another run was made at 2100 GMT which shows results very similar to that for 1834 GMT. The slope change that appears at 1955 GMT may also be due to a relatively large N change and no change in G. This effect can be seen by comparing the postulated G change with the receiver AVC (average signal strength) (see Fig. 4). The increased absorption at 2000 GMT would cause some slope change in the interaction coefficients; hence G will not be as high as the first estimate that was made assuming no large N change.

The conclusion is that if there is a G change it is probably no more than $0\cdot3\times10^{-3}$. This would imply, using the $T^{-5/2}$ temperature dependence, a D-region temperature drop of about 10 K.

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Response of the F-region Ionosphere to a Solar Eclipse

MEASUREMENTS of Faraday rotation of a linearly polarized signal have been used widely as a means of obtaining the integrated electron density or electron content in the ionosphere. Early measurements used the Moon as a reflector of radar signals and hence provided data only when the Moon was above the horizon. Faraday rotation measurements have also been carried out during a solar eclipse[1-3]. We report here some preliminary results of the Faraday rotation technique during the March 7, 1970, solar eclipse, using signals transmitted by the geostationary satellite ATS-3 at 137 MHz. Continuous observations were made at Norfolk, Virginia (36·56° N, 72·24° W), Turkey Point, Florida (29·95° N, 84·50° W), and Urbana, Illinois (40·069° N, 88·225° W). During the eclipse the satellite was located at 85·5° W. There are differences in the results from these three stations, but only the Norfolk data are presented here. The location of the 350 km subionospheric point at Norfolk is 33·87° N, 77·07° W.

The Faraday rotation measurement by a polarimeter gives only the relative change in polarization. We assume the orientation of the E-plane transmitted by ATS-3 to be 20° with respect to the spin axis, as measured by Klobuchar (unpublished). Our past experience agrees with this value. The daytime content is not very sensitive to the exact value of the transmitted polarization, however.

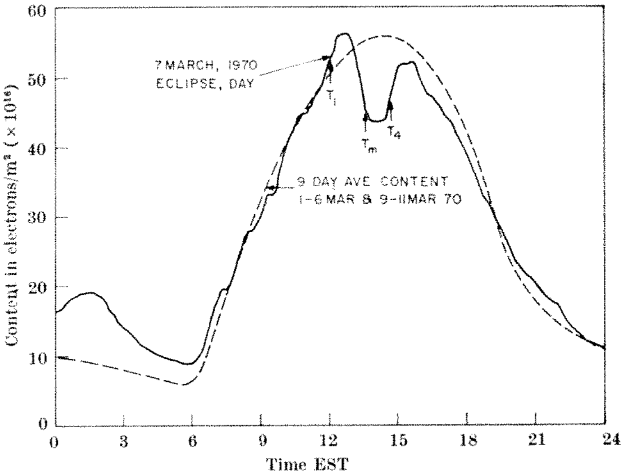

Fig. 1. Eclipse day and control day variation of electron content at Norfolk, Virginia.

Fig. 1 shows the average diurnal variation as well as the eclipse day variation in electron content. The average curve is the mean for the period March 1–11, 1970, excepting the values for March 7 and March 8 (March 8 was very disturbed). The first contact T_1 of the eclipse was at 1206 EST, and the content continued to increase for approximately 40 minutes after T_1 before it started to decrease when the Sun was approximately 40 per cent eclipsed. The minimum in content was fairly broad and was reached 39 minutes after totality, denoted by T_m. The content returned to its normal value about 43 minutes after fourth contact T_4. The change in content from the first maximum to minimum was $12\cdot8\times10^{16}$ electrons m^{-2}, or a decrease of 22·5 per cent. The change in content from the minimum to the second maximum was $8\cdot5\times10^{16}$ electrons m^{-2} or an increase of 19·5 per cent. These percentage changes are in agreement with those measured by Evans[4] for a 95 per cent eclipse in 1963, although the absolute values are quite different because of a difference in solar activities. The magnitude of the change reported here is four to five times larger than those observed by Evans for solar minimum conditions.

To simulate the eclipse behaviour of the F2-region, we have solved numerically the ionization equation of continuity and the neutral equation of motion. Detailed discussion of these equations, the neutral atmosphere model and ionospheric processes under normal conditions can be found elsewhere[5]. Here we will discuss only the perturbation introduced during the eclipse, and the results of computations.

The proportion of the solar disk unobscured by the Moon during an eclipse is given by the eclipse function $E(t)$ which is approximately V-shaped and is nearly linear for each segment. For simplicity, we approximate $E(t)$ by two linear curves. The eclipse function is then completely defined by specifying the time of first contact T_1, the time of maximum obscurity T_m, the time of fourth

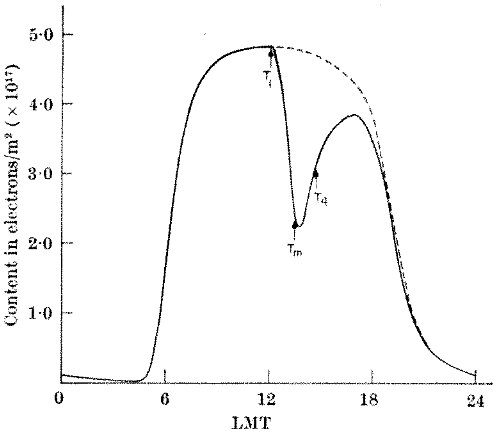

Fig. 2. Computed variation of electron content on an eclipse day.

contact T_4 and the percentage of maximum obscurity. Under the assumption that the Sun radiates uniformly across the solar disk, the intensity of the solar EUV radiation at the top of the atmosphere in the eclipse shadow region is given by $S'_\infty(t) = S \cdot E(t)$ where S_∞ is the intensity of solar EUV radiation under normal conditions. Because the production function of ionization is proportional to the intensity of the solar EUV radiation, the production function P' during the eclipse is given by $P'(t) = P \cdot E(t)$.

At present the precise variation of the exospheric temperature in the eclipse shadow region is not available although some rough experimental estimates have been made[4]. We have assumed arbitrarily that the decrease of the exospheric temperature is proportional to the eclipse function, and that the maximum exospheric temperature drop ΔT is 10 per cent of the minimum night-time temperature T_0 provided by Jacchia's model of the neutral atmosphere. The theoretical computations (ref. 6 and Shimazaki at the IAGA Scientific General Assemblies, 1969) have shown consistently a time delay between the time of maximum energy input and the time of maximum temperature of the atmosphere, so we also introduced a one hour time delay Δt between the time of maximum obscurity and the time of maximum temperature drop. The perturbed exospheric temperature in the eclipse shadow region is then given by $T'_\infty = T_\infty - \Delta T \cdot E(t - \Delta t)$. Here T_∞ is the exospheric temperature under normal conditions. The perturbed electron and ion temperature profiles in the shadow region are assumed to be

$$T'_e = (T_e - T'_n)\ \mathrm{Ch}(t)\ .\ E(t) + T'_n$$
$$T'_i = (T_i - T'_n)\ \mathrm{Ch}(t)\ .\ E(t) + T'_n$$

T_e, T_i are the temperature profiles for electrons and ions under normal conditions, T'_n is the perturbed neutral temperature profiles and $\mathrm{Ch}(t)$ is the normalized simple Chapman function. There is no time delay introduced for the temperatures of charged particles. A dynamo electric field given by Maeda and Kato[7] is also included in the computation. The dynamo field is assumed to be unaffected by the eclipse although there is evidence for a non-negligible effect[8].

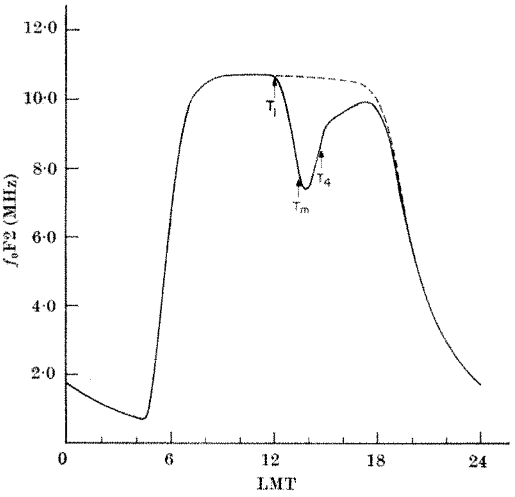

Fig. 3. Computed f_0F2 variation on an eclipse day.

Computations have been made for a station at 33·87° N, 77·07° W and a magnetic dip angle of 67°. The 10·7 cm solar radiation intensity used in the computation is 310×10^{-22} W m^{-2} Hz^{-1}. The computed variations of electron content, f_0F2 and neutral wind velocity vector at a height of 400 km are shown in Figs. 2–4 respectively.

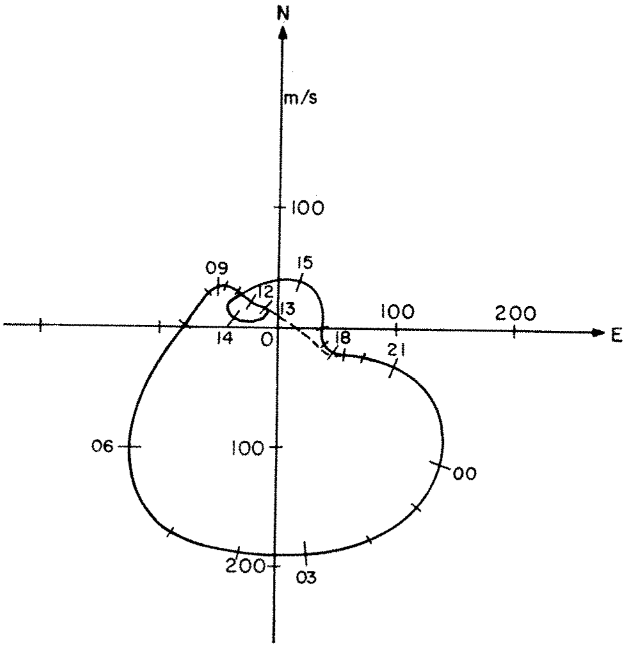

Fig. 4. Locus of wind velocity vector as a function of time at a height of 400 km on an eclipse day.

By comparing the computed variation of electron content with the data obtained at Norfolk we find that: (1) The general shapes of the computed and observed content curves in a normal day are somewhat different. The computed electron content increases rapidly during sunrise, reaches a plateau and stays relatively unchanged until sunset when the content begins to decrease very rapidly. But the observed content continues to increase with a constant rate after sunrise, reaches a maximum at about 1400 LMT, and then begins to decrease. (2) The computed electron content responds to the eclipse almost instantaneously and decreases much faster than the observed values. The observed content begins to decrease about 40 minutes after the beginning of the eclipse. (3) The predicted decrease in electron content during the eclipse is about $2 \cdot 3 \times 10^{17}$ m^{-2}, but only about $1 \cdot 3 \times 10^{17}$ m^{-2} in the observed data. (4) The time when the content reaches a minimum lags behind the time of maximum obscurity by about 25 minutes in the computations, while the observed time lag is about 45 minutes. (5) On the eclipse day the wind is quite different from the wind on a normal day.

The differences are difficult to reconcile. The eclipse effect on the electric field has not, of course, been taken into account. Other factors may have to be modified as more eclipse data become available.

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Possible Detection of Atmospheric Gravity Waves generated by the Solar Eclipse

Chimonas and Hines recently suggested that the cool shadow of the Moon, moving at supersonic speed across the atmosphere of the Earth during a solar eclipse, should generate gravity waves which would build up into a bow wave[1]. They estimated a maximum pressure perturbation (at a distance of 10,000 km from the shadow) of 10^{-5} at ground level and 10^{-1} at 200 km altitude. The high altitude effect should leave an ionospheric signature in the form of a travelling ionospheric disturbance (TID).

Our group at Stanford has been studying the columnar electron content of the ionosphere for a number of years by measuring the Faraday rotation angle of the VHF transmissions from such geostationary satellites as ATS-I and ATS-III (ref. 2). These measurements have been made at a number of locations in North America. At the time of the solar eclipse of March 7, 1970, four stations were in operation in the western United States.

The gravity waves generated by the eclipse of March 7 should reach California some time after 1900 UT on that day according to the prediction of Chimonas and Hines. Fig. 1 shows the fluctuations in electron content observed along the Stanford to ATS-III and Stanford to ATS-I paths near that time. The 300 km ionospheric points of these paths are separated by about 600 km. The fluctuations were determined by subtracting a 31 min running mean from the observed electron content. The time periods preceding and following the interval depicted in the figure were characterized by very little fluctuation so that the undulations constitute a well isolated event occurring at about the time when the gravity wave was expected.

The two trains of oscillations, although strongly correlated, are not entirely similar, so that the association of peaks in one train with those in the other is a somewhat subjective matter. If the matching suggested by the letters in Fig. 1 is accepted, then, because ATS-III is east of ATS-I, the oscillations can be interpreted in terms of a disturbance travelling east to west. Electron content records from Fort Collins, Colorado (with the 300 km ionospheric point 1,280 km to the east of that on the Stanford ATS-I path), and Clark Lake, California (300 km ionospheric point 600 km south-east of that of Stanford), show disturbances similar to those in Fig. 1. The Clark Lake record is of poor quality, however. Estimates of the time shifts between similar features observed at any three of the four stations allow the horizontal phase velocity of the disturbance to be determined. The geometry is such that it is essential that the Clark Lake information be used, however, because the remaining sub-ionospheric points are nearly in a straight line.

A peak thought to be the same as that labelled *b* in Fig. 1 was observed at 1910 UT at Fort Collins and at 1928 UT at Clark Lake. Assuming that the bumps are matched correctly, the uncertainty in the time of the peaks at Stanford and Fort Collins is ±2 min, and the uncertainty is ±3 min at Clark Lake. Using these times and uncertainties, the horizontal phase velocity of bump *b* is 620 ± 120 m s^{-1} at a bearing of $279 \pm 25°$ east of north. (The direction given is that of the velocity vector.) Velocities computed for the other bumps are roughly the same. The maximum possible speed for *b* based on the Stanford ATS-I and Stanford ATS-III records is substantially lower, however.

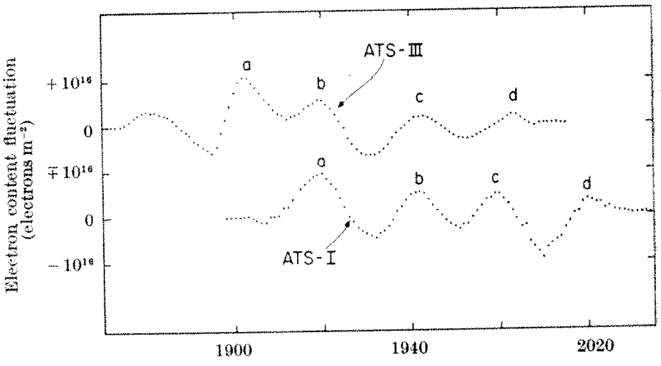

Fig. 1. Fluctuations in electron content along the Stanford to ATS-III and Stanford to ATS-I paths on March 7.

The amplitude of the undulation observed by the two Stanford stations is about $\pm 10^{16}$ electrons m^{-2}, and the background electron content amounted to some 60×10^{16} electrons m^{-2}. The relative amplitude of the undulation was therefore of the order of 1·5 per cent. The corresponding disturbance in the neutral atmosphere (if indeed the fluctuations observed were due to such a disturbance) may have been much larger because the direction of propagation, being roughly perpendicular to the geomagnetic field lines, may have resulted in a weak coupling between the atmospheric waves and the ionization. Disturbances travelling from east to west and having a similar character over the extent of the network (more than 1,000 km) have not been observed previously, although such disturbances travelling from north to south are often seen during geomagnetic storms[3]. The fact that these disturbances, of a rather unusual nature, occurred near the time predicted for arrival of atmospheric gravity waves due to the eclipse and that they came from the direction of the path of the eclipse implies a possible confirmation of Chimonas and Hines's suggestion.

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Eclipse Observations at Arecibo, Puerto Rico, on March 7, 1970

THE data in this letter were gathered at Arecibo Observatory[1] using the incoherent backscatter technique[2]. Observations were made during the March 7, 1970, eclipse and on March 6 and March 8 as control days. The observations were made in two modes summarized in Table 1: one mode combining plasma line and single pulse autocorrelation data (unpublished work of Harper), the other using double pulse techniques[3] for improved altitude resolution in the bottomside ionosphere. The eclipse reached a maximum of about 30 per cent near 1430, with a semiduration of about 80 minutes.

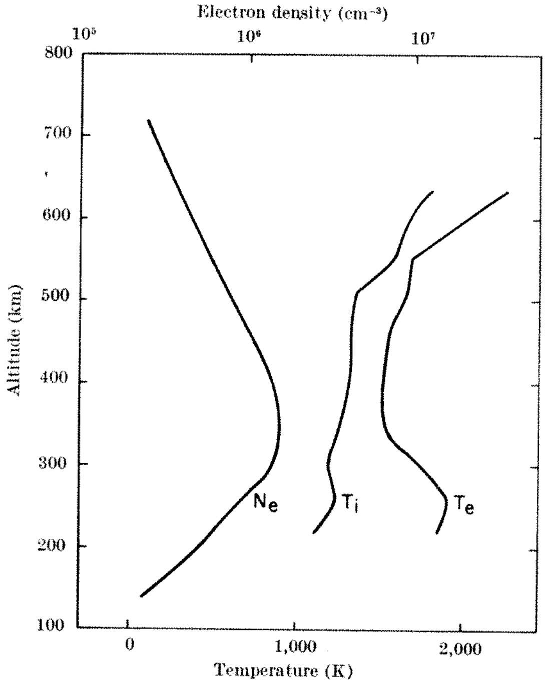

Fig. 1. Pre-eclipse electron density (N_e), ion temperature (T_i) and electron temperature (T_e) profiles for Arecibo Observatory, 1258 AST to 1313 AST, March 7, 1970.

Profiles of electron density N_e, electron temperature T_e and ion temperature T_i taken near the beginning, middle and end of the eclipse are shown in Figs 1, 2 and 3 respectively, as obtained in the plasma line mode. There are no striking effects in the temperatures. Apart from the post eclipse T_i at 500 km, the ion temperature profiles agree below 600 km within their 5 per cent error bars. The mid-eclipse T_e profile is systematically about 100 K lower than the other two profiles. But because density-temperature coupling has often led to T_e changes at Arecibo exceeding 1,000 K with negligible changes in the heat input, the question of eclipse effects on T_e must be viewed in terms of energy input. The plasma line intensities (yielding the derived parameter $KT_{\parallel}$) contain information regarding the photoelectron flux[4], but these intensities show no significant change during the eclipse.

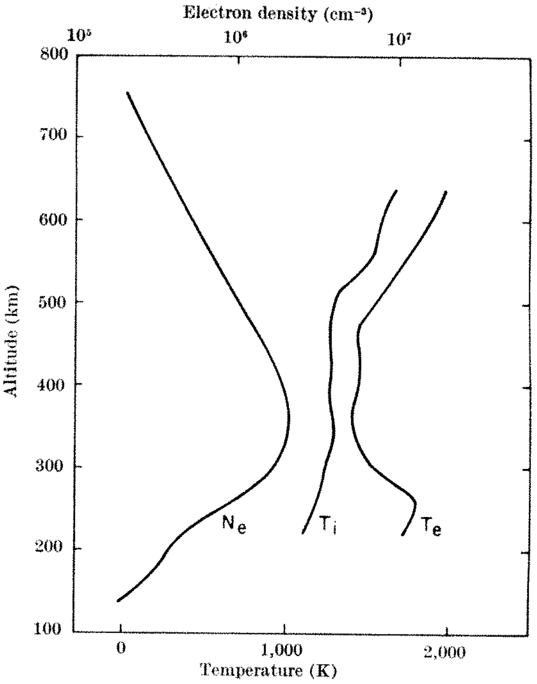

Fig. 2. Mid-eclipse electron density (N_e), ion temperature (T_i) and electron temperature (T_e) profiles for Arecibo Observatory, 1418 AST to 1433 AST, March 7, 1970.

Estimates of the electron-to-ion energy transfer rate

$$L_{ei} = 4{\cdot}8 \times 10^{-7}\ T_e^{-3/2}\ (T_e - T_i) N_e N_0^+ \ (\text{eV cm}^{-3}\ \text{s}^{-1})$$

give 10,000, 6,760 and 10,230 (±15 per cent) eV cm^{-3} s^{-1} for the pre-eclipse, mid-eclipse and post-eclipse periods respectively at 300 km; and 4,900, 3,340 and 6,600 (±15 per cent) eV cm^{-3} s^{-1} respectively at 260 km. This reflects a decreased heat input to the electrons in this bottomside altitude range of roughly 30 per cent during mid-eclipse, matching well to the percentage of totality experienced. These 15 per cent error bars allow the neglect of other terms, pending the full heat balance calculation including conduction and loss to molecular and atomic species. The heat conduction

$$Q_c = 7{\cdot}7 \times 10^5 \sin^2 I\ T_e^{5/2}\ \frac{dT_e}{dh}\ (\text{eV cm}^{-2}\ \text{s}^{-1})$$

downward through the 550 km level throughout the eclipse is consistent with $1{\cdot}8 \pm 0{\cdot}3 \times 10^9$ eV cm^{-2} s^{-1}. The heat conduction downward through 600 km was decreased at mid-eclipse by an amount that seems to be statistically significant, but could be explained simply by the increased topside electron density and the consequent electron-to-ion loss rate at this time.

The bottomside electron density profile decreased from the pre-eclipse to the post-eclipse period by an amount appropriate to the change in solar zenith angle; the bottomside mid-eclipse electron density is substantially

Table 1. SUMMARY OF THE ARECIBO OBSERVATIONS

Parameters measured	Techniques PL	PP	AC	Altitude resolution	Time resolution	Uncertainty	Measured range Top and bottomside	
N_e	X			2–5 km	7 min	±2 km	3·5–8·5 MHz	Mode 1
$KT_{\parallel}$	X			~5 km	15 min	~30%	~5 –25 eV	
T_e			X	40 km	7 min	6%	220–640 km	
T_i			X	40 km	7 min	6%	220–640 km	
N_e		X	X	<45 km	7 min	~5%	220–640 km	
T_e/T_i	X	X	X	40 km	7 min	~5%	130–780 km (PP) 3·5–8·5 MHz	
N_e		X	X	9 km	12 min	~6%	100–500 km	Mode 2
T_e		X	X	9 km	12 min	~6%	115–300 km	
T_i		X	X	9 km	12 min	~6%	115–300 km	
%O^+		X	X	9 km	12 min	~10 km	145–235 km	
Vel (F)		X	X	9 km	12 min	~20 m s^{-1}	115–300 km	

PL: plasma line; PP: power profile; AC: autocorrelation function.

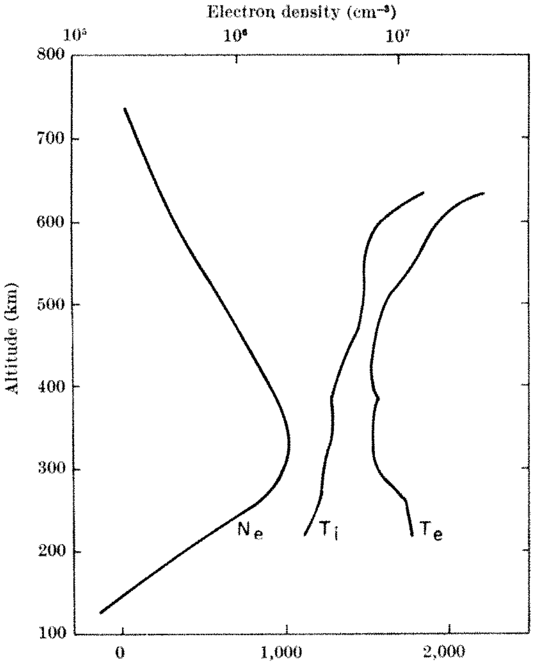

Fig. 3. Post-eclipse electron density (N_e), ion temperature (T_i) and electron temperature (T_e) profiles for Arecibo Observatory, 1525 AST to 1540 AST, March 7, 1970.

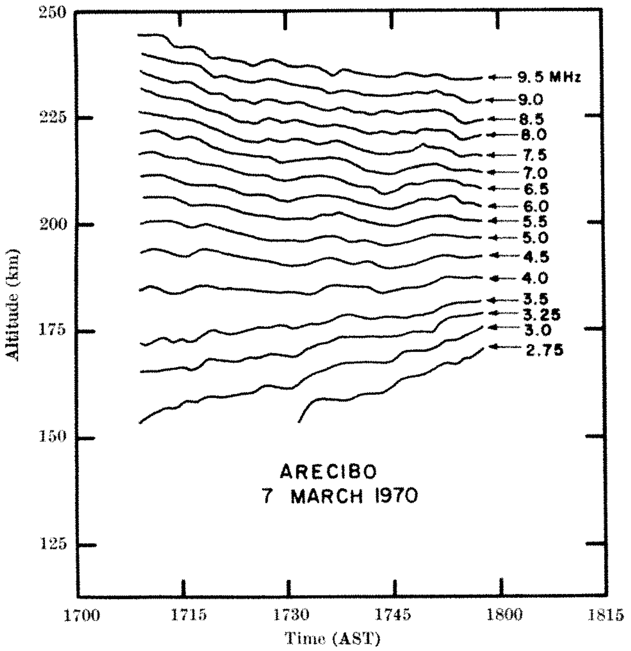

Fig. 4. Contours of constant backscatter power versus altitude and time. Transmitted pulse length = 300 μs; time smoothing ≈2 min. AST = GMT − 4 h.

lower near the E-region as recombination effects would lead one to expect. The ionosonde showed a distinct F_1 ledge (also visible in Fig. 2) for about 45 minutes centred on the eclipse, contrary to the normal situation at this time when there is usually only the mere suggestion of a ledge. The implications to ionospheric chemistry of this and of the mode 2 data are yet to be studied.

The topside electron density at mid-eclipse is seen to be substantially increased. It remains to be seen whether the mechanism for this increase is a decreased topside T_e and downward transport from well above 700 km, or an upward transport of the F-layer profile (vertical displacements of 40 km over time scales of the order of an hour are not uncommon, particularly on magnetically disturbed days).

It was suggested by Chimonas and Hines[5] that atmospheric gravity waves might be generated by a solar eclipse. Contour plots of constant backscatter power reveal a weak wave-like disturbance between 1725 AST and 1800 AST. Fig. 4 shows the contours for the time before and during the disturbance. (The subsequent section of data showed that the disturbance ceased about 1800 AST.) The values of the contours are normalized to the F-region critical frequency determined from an ionogram. The wave structure is apparent in the altitude region between the 3·5 and 8·0 MHz contours, that is, between 175 and 225 km, and the period is 15 minutes. At 215 km, the amplitude of the fluctuations in backscatter power is 6 per cent, which is four times the standard deviation of the measurement. If it is assumed that the disturbance originated from the point of closest approach of totality, then the velocity of propagation would be 200 m s⁻¹. The question of whether these waves are associated with the eclipse needs to be considered within the context of data from other stations.

It should be noted in passing that the data for March 8 are of little value as a control in view of the very high magnetic activity on that day.

This research was supported in part by a US Air Force Office of Scientific Research grant to Rice University. The Arecibo Observatory is operated by Cornell University under contract to the National Science Foundation and with partial support from the Advanced Research Projects Agency. We thank the staff of the Arecibo Observatory and Dean W. E. Gordon for their support.

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Travelling Ionospheric Disturbances in Athens during the March 7 Solar Eclipse

Chimonas and Hines[1] point out that a solar eclipse, by interfering with the heat balance in the shaded portion of the atmosphere, can be expected to generate internal gravity waves, which should be detected at some considerable distance from the eclipse path.

Measurements in the Ionospheric Institute of the National Observatory of Athens several days before, after and on the eclipse day show some unusual variations of f_0F2 and of *h* min.F. Fig. 1 shows unusual increases in f_0F2 at 1900 and 2300 UT of March 8, and also an unexpected deep decrease at 0500 UT on March 9. The corresponding continuous record of *h* min.F for the same period indicates a strong variation between 0300 and 0600 of March 9.

Observations made by the National Meteorological Service of Greece indicated a variation of the order of 0·1 mbar between 2300 and 2400 UT on March 7.

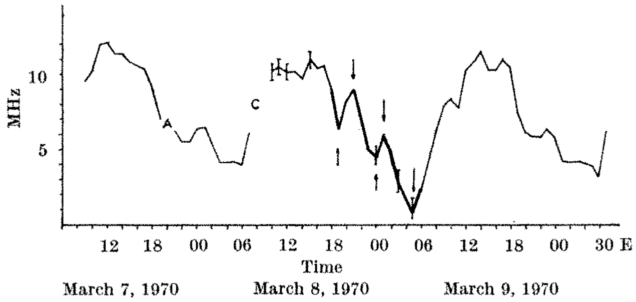

Fig. 1. Variation of f_0F2 in Athens during the period before, after and on the eclipse day.

Any attempt to associate these observations in Athens with the total eclipse over North America and Canada is premature. Only systematic observations from ionosondes and microbarographs around the world would justify the conclusion that the travelling disturbances were the result of an eclipse effect.

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VLF Radio Signals observed in Newfoundland during the Solar Eclipse of March 7, 1970

THIS is a preliminary report on some naturally occurring radio signals observed in the very low frequency (VLF) band between 1 and 10 kHz during the solar eclipse of March 7, 1970. The observations were from a site close to the STADAN station near St Johns, Newfoundland, Canada, at geographic latitude 47° 44·9′ N, longitude 52° 43·4′ W, $L = 3{\cdot}46$. They formed part of a joint experiment in which VLF signals were also received in the magnetic conjugate area, at Halley Bay, Antarctica, and at a lower latitude, at Norwich, Vermont, USA.

The aim of the experiment was to record whistlers[1] during the eclipse period, and to search for effects which might indicate changes in the distribution of magnetospheric plasma along tubes of force of the geomagnetic field[2]. Unfortunately whistlers were not received in Newfoundland between 1800 and 2000 UT during the eclipse, although they were recorded during this interval on the control days and after 2030 UT on the eclipse day. Some interesting phenomena were found, however.

We used a single-turn, square loop antenna, of side 3 m, mounted with the plane of the antenna vertical and in the magnetic east–west direction. With this orientation the antenna did not respond to signals propagating along the magnetic north–south meridian. The path of the eclipse near Newfoundland was nearly parallel to the isoclinic lines, so that whistler-mode signals propagating along field lines with their "feet" in the eclipsed ionosphere were received preferentially. The signals were then amplified by a battery-powered, low-noise transistor amplifier, and signals from radio transmitters were removed by an *LC* low-pass filter tuned to 18 kHz. Twin-T band-reject filters at 16, 17·8, 100 and 112·6 kHz removed strong signals from GBR, NAA, Loran and a local navigation station respectively. Although the equipment was set up in a clearing in the forest some 2 km from the STADAN station, it was necessary to use two similar filters at 180 Hz to reduce interference associated with power-lines to an acceptable level. The amplified VLF waveform was recorded on magnetic tape. The intensity of the background signal at two frequencies, 2·90 and 5·36 kHz,

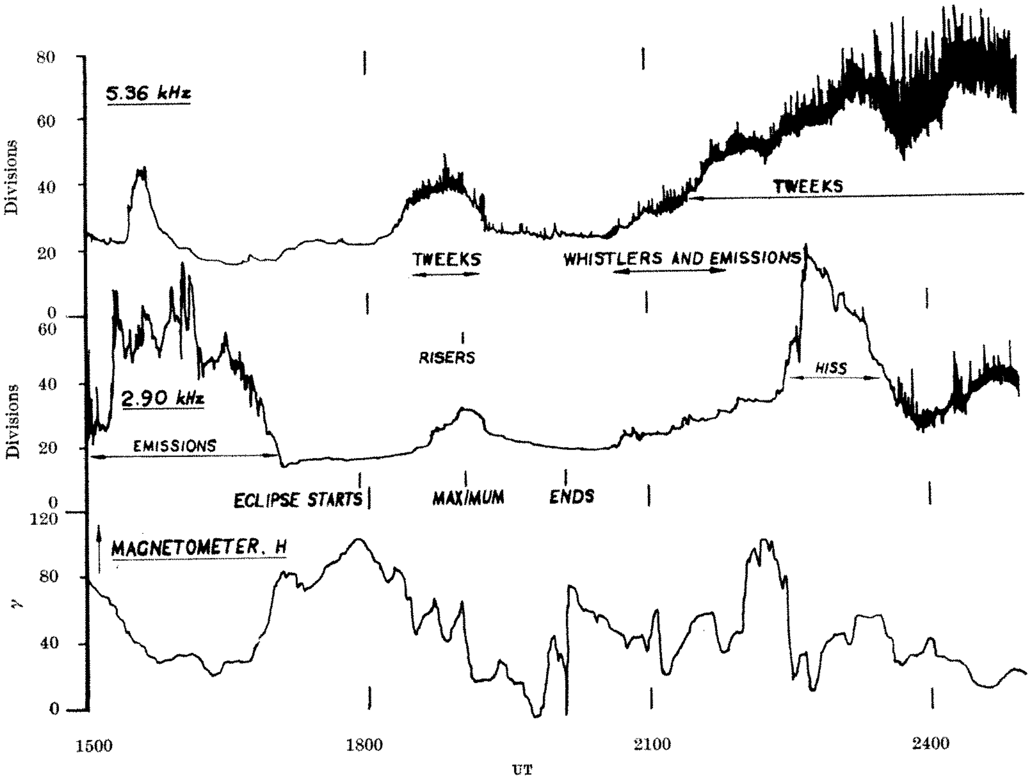

Fig. 1. Upper trace: the intensity of the background signal at 5·36 kHz, showing the occurrence of tweeks[4] during the eclipse and during the local night, each vertical trace corresponding to a particularly strong atmospheric. Middle trace: the intensity of the background signal at 2·90 kHz, showing the occurrence of chorus emissions before 1700 UT, of risers between 1901.31 and 1903.42 UT, and of hiss between 2230 and 2330 UT. Lower trace: the north–south horizontal component of the geomagnetic field, 1 γ being 1 nanoTesla. All quantities are increasing upwards.

was observed using a three-channel recorder, the paper chart moving at 2·5 cm h^{-1}.

A circuit, based on that of Ellis[3], minimized the effects of atmospherics[1]—energy radiated by lightning discharges. Fluctuations in the north–south horizontal component of the Earth's magnetic field were also recorded, using a flux-gate magnetometer.

Fig. 1 shows the chart recording for the 10 h period from 1500 UT on March 7, 1970. Geomagnetic activity during this interval was high, and from this record alone there is no definite evidence for a decrease in the magnetic field that could be attributed to a change in currents flowing in the dynamo layer of the ionosphere[4]. Before 1700 UT, VLF emissions of the chorus[1] type were recorded. These are particularly strong in the 2·9 kHz band, as they are at South Uist, Outer Hebrides, Scotland, at a similar magnetic latitude. There is also some energy at the higher frequency. After 2100 UT (1730 local time), the predominant signals were tweeks[1], which are atmospherics that have propagated for a considerable distance in the waveguide formed by the Earth and the night-time ionosphere.

Some 30 min after the eclipse starts (first contact was at 1755 UT) the intensity of the signals in both VLF channels increased. Tweeks were heard for nearly an hour, indicating that electrons in the lower ionosphere quickly recombined, the electron density falling to a post-sunset value. Night-time propagation conditions existed. After the maximum eclipse effect, the eclipse being total in the ionosphere above the receiving station, daytime conditions return for an hour and a half before sunset.

An unexpected phenomenon was observed at the time of totality in the ionosphere. A sequence of intense, rising-frequency, musical tones, known as risers[1], was recorded. Fig. 2 shows the frequency-time spectrogram of one such riser, received at 1902·41½ UT, rising from a frequency of 1·8 kHz to 2·4 kHz in $\sim$75 ms. The strong vertical lines are atmospherics which contain little energy below 4 kHz. The strong horizontal line at 10·2 kHz is due to an Omega transmitter. Some weak horizontal lines below 3 kHz, which are associated with harmonics of the fundamental frequency of the power-line signal, are also apparent.

Eighteen intense risers of this genetic type were recorded within 2¼ min, and their distribution in time is shown in Fig. 3. Three somewhat weaker, but otherwise similar, risers were received earlier, between 1827 and 1837 UT. One was received at 1845·37 and four more between 1847·53 and 1848·14 UT. With one exception, all these risers occurred at times when the strength of the magnetic field was rapidly decreasing.

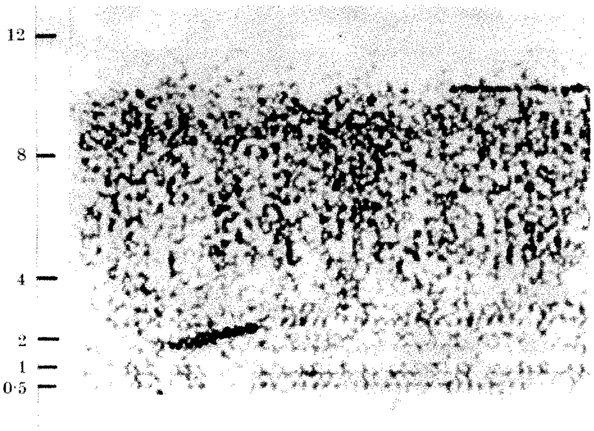

Fig. 2. Spectrogram of VLF waveform at 1902·41½ UT, showing a riser ($\sim$2 kHz), atmospherics, and an Omega transmission.

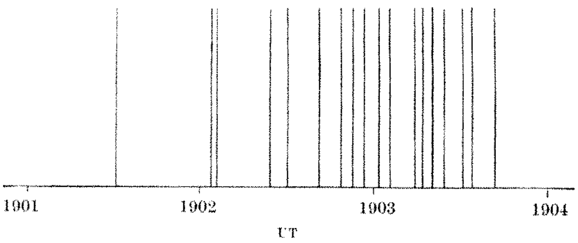

Fig. 3. Diagram showing times of occurrence of risers.

It is probable that these risers are produced by a transverse plasma instability in the magnetosphere (unpublished work of Burtis), in which a whistler-mode signal grows in intensity under conditions of enhanced magnetic activity and during the local afternoon. We suggest that they are received on the ground only at times close to eclipse totality because of the then much-reduced electron density—and hence absorption—of the D-region. An alternative explanation is that as the whistler-mode ray propagates through the ionosphere, its wavenormal is scattered downwards by the large horizontal gradients of electron density existing in the vicinity of the eclipse-induced "hole" in the ionosphere. The ray would then not be reflected as it would be by the undisturbed ionosphere. A contributory factor is the considerable attenuation suffered by waves of this frequency when propagating in the Earth–ionosphere waveguide[5], $\sim$30 dB/1,000 km at 2 kHz.

Strong whistlers were recorded between 2030 and 2200 UT. During this interval, some peculiar VLF emissions, with both rising and falling frequency-time characteristics, were received. For an hour from 2230 UT the dominant signal at 2·9 kHz had mainly the characteristics of hiss[1]. Analyses of these data, and of those obtained during the magnetic storm of March 8, 1970, are continuing.

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VLF Propagation Effects produced by the Eclipse

Very low frequency (VLF) transmissions from three locations (Omega, Haiku, 10·2 kHz; NLK, Seattle, Washington, 18·6 kHz; and NAA, Cutler, Maine, 17·8 kHz) were received at Umuarama Radio Observatory, Campos do Jordão, São Paulo, Brazil, during the total solar eclipse of March 7, 1970, by two receiving stations controlled by a rubidium oscillator. The phase outputs had widths of 0–100 μs (± 1 μs) and 0–10 μs (± 0·1 μs), and the recording time constants were 50 s in phase and in amplitude. The propagation paths were 12,900 km, 10,900 km and 7,900 km long respectively (see Fig. 1).

Fig. 2C shows the effect on the NAA–São Paulo path. The dashed curve shows the average phase change on a

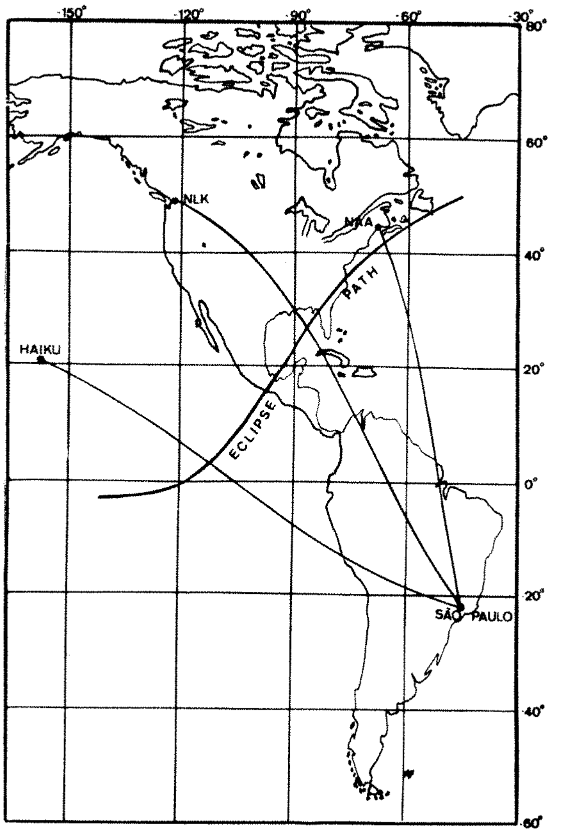

Fig. 1. The geometry of the propagation paths from Haiku, NLK and NAA to São Paulo and the path of totality of the eclipse.

normal day and the full curve the phase changes observed on the day of the eclipse. The times of first contact, maximum phase at ground level, and fourth contact are marked. The maximum phase delay was 4·5 μs which occurred nearly five minutes after totality crossed the path. The time interval between the onset of the phase retardation and first contact over the path was eleven minutes.

Two important points should be mentioned. (1) Because the VLF station at Campos do Jordão comprises only two receivers, the following tracking procedure was adopted (see Fig. 1). Stations Haiku (10·2 kHz) and NLK (18·6 kHz) were monitored until 1730 UT when the Haiku receiver was switched over to receive NAA. Monitoring of NLK continued uninterrupted. (2) A sudden ionospheric disturbance (SID) which occurred at 1605 UT affected the phase and amplitude of both Haiku and NLK, but of course was not recorded on the NAA–São Paulo path. We feel that the time difference of eleven minutes between first contact and the change in phase on NAA, which is much smaller than the 30–45 minutes reported in other observations (unpublished work of Hildebrand, and ref. 1), is a direct consequence of the SID.

The changes in amplitude, not shown in Fig. 2, were in general small, the maximum increase being less than 0·3 dB.

Fig. 2B shows the phase changes on the NLK–São Paulo path, and the effect of the SID can be seen clearly. First contact occurred at 1643 UT, during the recovery period of the SID. The time at which the phase change due to the eclipse began is marked as EEC, and was obtained by comparison of the phase and amplitude records. The amplitude had recovered to normal after the SID, and the commencement of the eclipse effect could be seen clearly. The maximum phase change was about 4·5 μs and the delay between phase maximum and totality over the path was about nine minutes. Amplitude changes were once again very small, the increase being nearly 2·4 dB.

Unlike the NLK and NAA paths which were fully sunlit throughout, the eclipse crossed the Haiku–São Paulo path during sunrise. The dashed line in Fig. 2A represents the normal average phase transition observed during sunrise, and the full line curve represents the phase changes on the eclipse day. The SID occurred 45 minutes after first contact. It is interesting that the effect due to the eclipse (EEC in Fig. 2A) had already begun before the SID occurred. It should be possible in principle therefore to determine the location of the active region on the Sun responsible for the X-ray emission resulting in the SID. It is difficult to specify when the maximum phase change due to the eclipse occurred because of the SID, but the maximum marked S occurred ten minutes before totality.

Hildebrand (unpublished) carried out VLF measurements in southern Brazil at frequencies between 9·37 kHz and 31·25 kHz during the total solar eclipse of November 12, 1966. One set of measurements was at steep incidence and another set was on an oblique path along the path of the eclipse. The observations at oblique incidence show a phase retardation of about 6·0 μs at 31·25 kHz, falling to 1·0 μs at 15·6 kHz and again rising to 4·0 μs at 9·37 kHz. The phase retardation of 4·5 μs observed at 17·8 and 18·6 kHz in our experiments is comparable with the 3·0 μs retardation observed by Hildebrand at 18·75 kHz. The maximum phase retardation of about 5·0 μs observed at 10·2 kHz on the Haiku—São Paulo path compares well with that of 4·0 μs obtained by Hildebrand at 9·3 kHz. Kaufmann and Schaal[1] have also reported observations on a frequency of 26·1 kHz during the eclipse of November 12, 1966. They found a maximum phase delay of 12·3 μs when the path of totality crossed the propagation path. Signals at 16·0 kHz from Rugby observed at Mount

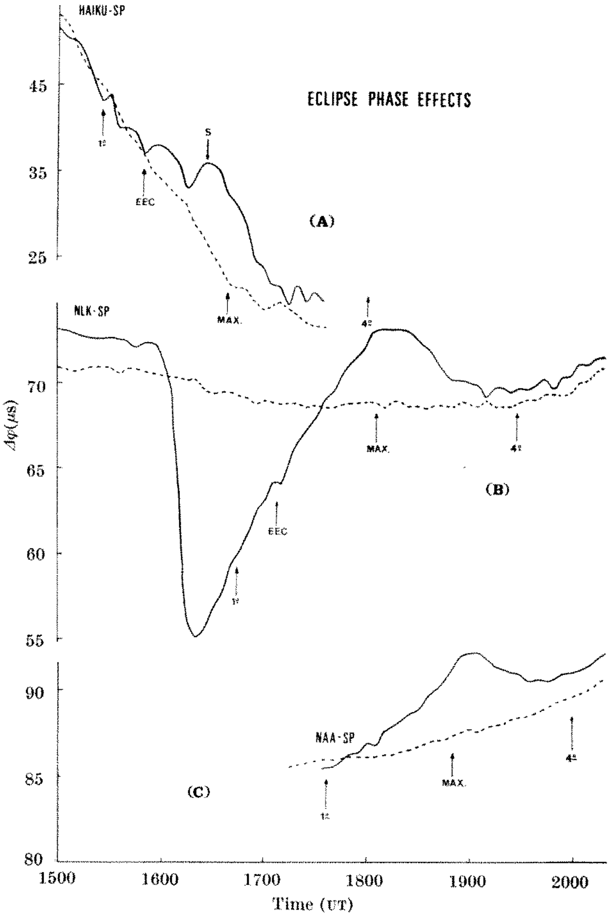

Fig. 2. Phase retardations for Haiku (A), NLK (B), and NAA (C) produced by the eclipse. The phase records for average normal days (dashed line) are also shown.

Stromlo, Australia, during the total solar eclipse of September 22, 1968, showed a phase delay of 3·0 μs (ref. 2). These observations seem to confirm Hildebrand's conclusion that there seems to be no distinguishable frequency dependence in the amount of phase advance.

The possibility of observing three VLF paths with two receiving stations was suggested by Dr F. H. Reder. This research was supported by the US Army Element Defense Research Office Latin America, Fundação de Amparo à Pesquisado Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisas (CNPq). We thank M. H. Paes de Barros, J. Teruo Ouchi and C. Monteiro for help.

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Comparison of 100 kHz Pulse Propagation during Two Solar Eclipses

IN July 1963 the path of a solar eclipse swept across the Aleutian Islands of Alaska. Low frequency (100 kHz) pulse transmissions of the US Coast Guard Alaskan Loran-C chain were observed in the one-hop sky-wave propagation mode for paths near regions of totality. This letter compares the data obtained during this eclipse[1,2] with results from the March 7 event.

In the 1963 data the phase of the ionospheric signal was retarded nearly in proportion to the increasing solar obscuration, indicating a maximum apparent reflexion height near maximum obscuration. Following the maximum obscuration, the phase advanced with an overshoot following the end of the eclipse (see Fig. 1). This pattern is similar to a change in ozone densities at heights above approximately 65 km calculated theoretically. The amplitude of the LF pulse signal increased rapidly near eclipse totality, and reached a maximim value 4 min after the time of totality. These changes are similar to calculated changes in the density of atomic oxygen for heights below about 65 km, except for the 4 min delay which was attributed to ionic composition changes that would take longer than 4 min if $[0] < 10^8$ atoms cm^{-3} during the eclipse.

During the March 1970 solar eclipse LF pulse observations were made over several paths of totality or near totality along the east coast of the United States, again using the Coast Guard Loran-C facilities. The midpoints of the paths were from 34° to 56° N latitude, and the path lengths were from 1,000 to 3,000 km. The behaviour during this eclipse was quite different from those observed in July 1963 (see Fig. 1 in which the March 1970 data (Dana, Indiana to Bermuda path) are typical of all paths). Rather rapid phase retardations were observed in the signals near the eclipse totality, with the maximum apparent height of reflexion occurring 5 min after totality. The amplitude change was small with the maximum signal level occurring slightly before totality. It should be pointed out that on March 7, 1970, a solar flare occurred less than 1 h before the eclipse started. This flare appreciably affected all the LF sky-wave signals and recovery was not fully achieved by the time the solar eclipse began as shown in Fig. 1. The flare may partly account for the amplitude maximum occurring before the time of maximum obscuration, but a complete explanation of this phenomenon is not easy.

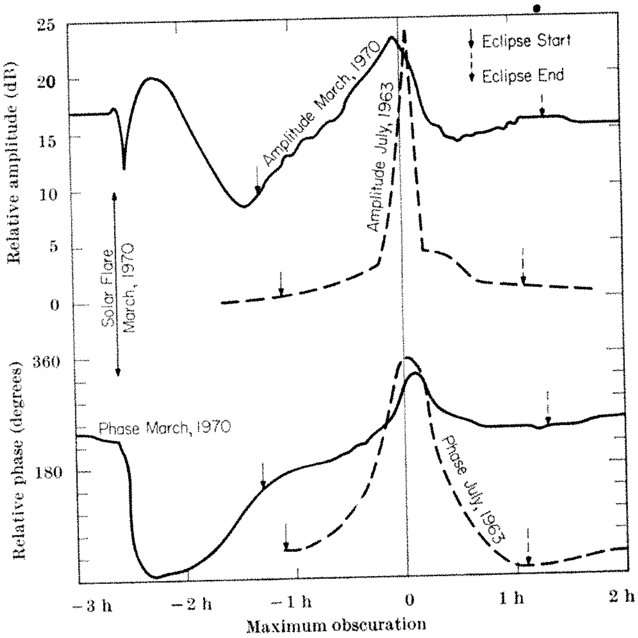

Fig. 1. Comparison of LF eclipse effects observed in July 1963 and in March 1970.

Aside from the problems produced by the solar flare preceding the eclipse, it is interesting to speculate on why the effects produced by the eclipse were so different in March 1970 and July 1963. The differences might have to do with the time in the solar cycle, different locations or path lengths for the measurements, or the season of the year. Both eclipses were near local noon, so the time of day should not be a factor. Routine LF sky-wave observations suggest that the propagation does not change appreciably with the solar cycle, apart from the effects of flares during solar maximum. The measurements in March 1970 covered several latitudes and several path lengths; if latitude or path length were a factor, the results from this eclipse should therefore have changed from path to path.

Seasonal changes in propagation do occur, however, and are similar in the United States and in the Aleutian Islands of Alaska. There is a lower reflexion height and greater ionospheric absorption in July than in March[3]. This could have to do with increased electron densities associated with increased production and concentrates of atomic oxygen during summer months, possibly by associative detachment of electrons from O_2^- by atomic oxygen. The daytime density profile of atomic oxygen would be expected to be lower in the summer than in March. Therefore the 5 min delay between the eclipse maximum and the phase peak observed in March could have been caused by the same mechanism that produced the delay of 4 min in the amplitude peak in the July measurements. If the apparent height of reflexion in March is determined by the density profile of atomic oxygen, few electrons would be produced by associative detachment by atomic oxygen at heights below this reflexion height because of rapidly decreasing atomic oxygen densities. Large amplitude changes, such as those observed in July, would not be expected to correlate with changes in the atomic oxygen density in this case.

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Received May 4, 1970.

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Preliminary Comparison of Predicted and Observed Structure of the Solar Corona at the Eclipse of March 7, 1970

RECENTLY Schatten predicted[1] the structure of the corona at the eclipse of March 7, 1970. The prediction was based on a projection onto the celestial plane of the coronal magnetic field resulting from a Green's function solution for the magnetic potential between the photosphere and a spherical equipotential "source surface" located 2 radii ($R_{\odot}$) from the solar centre. Smith, in conjunction with L. Weinstein, photographed the corona using a 90-inch focal length Newtonian telescope at the NASA–Langley Research Center in Hampton, Virginia. A rapidly rotating sector wedge occulter with radially varying aperture (radial transmission filter) was designed specifically for this telescope and located just before the focal plane to reduce the steep average gradient of coronal brightness before the film (unpublished work of Smith, Henderson, and Torrey). The radial variation of transmission from unity at 4 $R_{\odot}$ to 0·0025 at the solar limb greatly facilitates photography of fine and intermediate scale brightness structure without overexposure by the average corona. This letter describes the chief points of agreement and disagreement in the preliminary comparison of observation with prediction.

Three defects in the photographs, two of which are evident in Fig. 1, must be explained before examining the coronal structure. At the left (east) edge is a diffuse circular image of the bright inner corona caused by reflexion off an uncoated glass window supporting the secondary flat of the Newtonian system. Interpretation of apparent structure beyond 1·8 $R_{\odot}$ in this region can be misleading. Second, a circular band near 2·2 $R_{\odot}$ is due to a slight non-uniformity in the radial transmission filter. Its location indicates that the filter was centred on the coronal image to within 2 arc minutes. Finally, non-uniform motion of the shutter created a series of faint linear dark bands across the shorter exposures which is not evident on this long exposure.

The structure of the middle and inner corona shown in Fig. 1 is typical of Lockyear's maximum type corona[2] although the maximum smoothed sunspot number occurred some sixteen months earlier. Our classification of a maximum corona is based upon several structural features. First, the usual dipole-like plumes of the rotational poles have been replaced by streamers, arches, and linear rays. Second, the height above the limb of most closed structures is usually very low. Third, there is a predominance of open, linear, ray-like structure much of which has a roughly radial orientation. It is interesting that the solar magnetograph observations of Howard (unpublished) indicate that by March 7 the magnetic polarity at the rotational poles had not yet reversed. Thus, on the basis of the 22-year magnetic cycle[3], the maximum might not have arrived by the time of the eclipse. The apparent discrepancy is not too surprising, however, bearing in mind the rather flat maximum of the present sunspot cycle.

The observation is compared to prediction by reference to Fig. 2 and Table 1. Figs. 1 and 2 have been enlarged to the same scale and the rotational axis indicated on Fig. 1 is uncertain by less than ±1°. The first two columns of Table 1 describe the coronal structures observed and list the heliographic position angle (measured

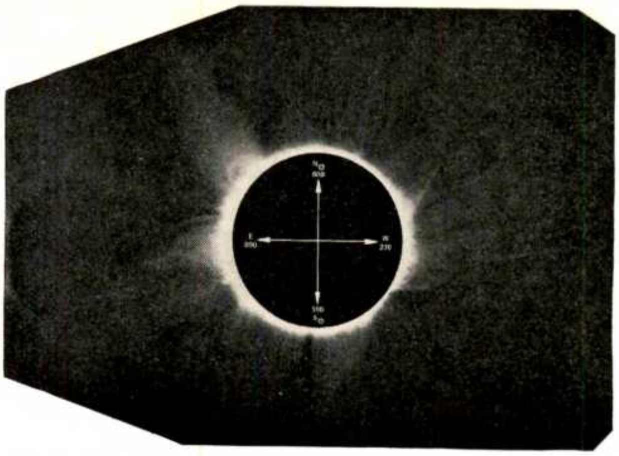

Fig. 1. The solar corona at 18 h 41 m 59 s UT on March 7, 1970. A radial transmission filter was employed to reduce the steep gradient of coronal brightness on this 2 s exposure. The Fluor-O-Dodge printer was also used to increase latitude in the reproduction process. A double reflexion exists at the east edge.

Table 1. COMPARISON AND PRELIMINARY EVALUATION

Observation		Prediction			
P.A.	Feature	P.A.	Feature	Comments	Preliminary evaluation
320–030	Many linear rays above low, elliptical arches	—	No prediction	North polar region: arch height 1/10 $R_{\odot}$ or less	—
031–069	Large helmet streamer (inverted "Y") over prominence at 052	030–070	Helmet streamers centred on 050	Maximum arch height of $\frac{1}{2}R_{\odot}$ agrees well with prediction	Clear agreement
069	Low, sharply cusped arches	075	Arch and ray	Arches are ~6° away	Qualified agreement
075	Short, narrow rays of a small condensation				
078–108	Non-radial active region streamers without helmet structure	100–120	Streamer without helmet structure	Partial overlap of features near limb, agreement improves with height	Qualified agreement
095	Small bright spot near the limb	095	Bright feature (condensation); small, tight arches	Condensations are + and − 20° away, although small arches could appear as a bright spot	Qualified disagreement
109–122	General region of condensation; central feature at 116	100–120	Streamers overlying arches at 100 and 110	An unusually high latitude condensation	Clear disagreement
123–148	Active region streamers and rays	120–150	Broad streamers without arches	Considered as a single feature for evaluation	Qualified agreement
—	—	145	Narrow streamer		
160–210	Broad streamer and helmet streamer (inverted "Y")	—	No prediction	South polar region: arch height $\frac{1}{4}R_{\odot}$ or less	—
210–236	Short, feather-like rays	210–230	Short rays	See Fig. 2	Clear agreement
		235	Narrow streamer		Qualified disagreement
236–268	Small helmet streamer (inverted "T") associated with prominence at 248	235–270	Broad streamer without arches	Prediction fails to include arches	Qualified disagreement
268–283	Faint, complex ray structure extending into mid-corona	278	Arch: rays in mid-corona	Except for the nested arches and prominence, unique identification of observed structure in this region is quite difficult. Arch height is near that predicted	Qualified agreement
288	Foot of a distinct arch and a small prominence	288	Bright feature (condensation)		Clear disagreement
292	Centre of symmetry of nested arches	292	Nested arches		Clear agreement
299	Long, narrow, radial ray; possibly a small condensation	—	—		—
283–297	Small helmet (inverted "Y") above nested arches; rays curve poleward	285–310	Helmet streamers centred on 302	Poleward curving features are considered as a single structure for evaluation	Qualified agreement
283–314	Poleward curving rays projected upon radial rays				

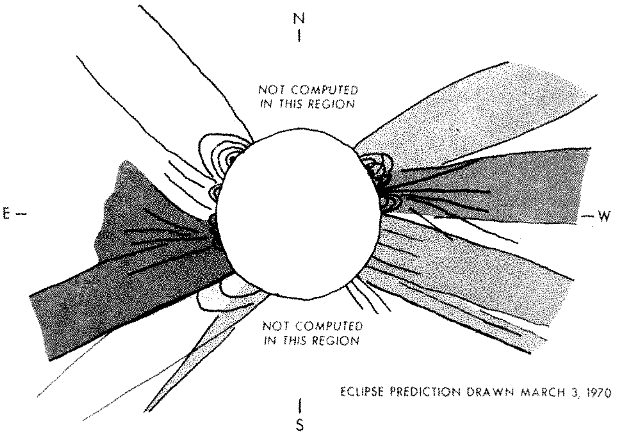
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Fig. 2. The structure of the solar corona predicted[1] for March 7, 1970.

counter-clockwise) of the intercept of those structures with the lunar limb. It should be pointed out that for a few non-radial, curvilinear structures of the middle corona this measurement procedure is somewhat artificial. The location at the limb of clearly identified features has been measured on contact positive copies with an r.m.s. error of 1·5°, which, when combined with the $\pm 2\frac{1}{2}°$ quantization interval of the calculation, leads to an uncertainty in the comparison of predicted and observed structures of ±3°. The descriptive terminology, inverted "Y" or "T", refers to the shape of helmet streamers as described in ref. 4. The next two columns of Table 1 give the position angle and description of the structure as described in ref. 1. The final two columns list pertinent comments and a preliminary evaluation of the prediction. "Clear agreement" means that the overall size, shape, and orientation of the structure predicted agree with that observed, in addition to the predicted location agreeing within the ±3° positional uncertainty of the comparison. "Qualified agreement" means that the prediction agrees approximately with observation, but that detailed agreement is lacking; or that the feature agrees well in structure, but the position disagrees by 3° to 6°. "Qualified disagreement" means that although the predicted structure may be nearby, it lies more than 6° away from the predicted position. "Clear disagreement" means a complete lack of positional and structural correlation.

In discussing this preliminary comparison, we first note that there are three cases of "clear agreement". It is particularly satisfying that the helmet at 052 degrees, the largest single structure visible at this eclipse, was correctly forecast. The maximum observed arch height agrees very closely with that predicted. The two cases of "clear disagreement" with the prediction are associated with the apparently erroneous location of coronal condensations which are usually considered to occur above active surface regions. It might appear that the location of the underlying active regions was not well known before the eclipse. That is not true, however, because such regions have a characteristic appearance on the magnetographic input data. Rather, something associated with the field calculations near active regions seems to be wrong. On the basis of experience with previous calculations and observations, we tentatively suggest that the source surface used in these calculations may have been located too high above the active regions. (We do not mean to imply, however, that by simple modification of the source surface one could incorporate the dynamic effects of solar flares into this fundamentally static calculation.)

Let us briefly examine this suggestion. First, use of a spherically symmetric source surface is clearly only an approximation because a spherically symmetric corona has never been observed. The structure seen at this eclipse is as good an example of this as any. Second, lowering the source surface above active regions will permit more magnetic field lines to leave the corona from lower heights than before and should reduce the size of the remaining closed structures. This is physically consistent with the generally higher temperature and density of active regions. Third, depressing the source surface should also reduce the somewhat excessive number of north–south oriented arches at present calculated near active regions because more of the adjacent, fringing field lines would then leave the inner corona rather than close downward. One observation at first seems to contradict this suggestion. The strength of the interplanetary magnetic field measured by spacecraft at 1 A.U. has not reflected the increased number of field lines to be expected from a reduced source surface height above the many active regions present at this maximum phase of the solar cycle. On the basis of Faraday rotation measurements of the field at 10 $R_{\odot}$, Schatten[5] has suggested that many of the field lines from active regions return to the Sun from the outer corona below the Alfven point near 30 $R_{\odot}$. Because such return does not influence the structure of the inner and middle corona, the spacecraft observations are not contradictory.

To summarize, the predominantly open structure of the predicted corona is in agreement on a large scale basis with the maximum type corona observed. The largest single structure apparent at the eclipse was correctly forecast. Detailed agreement of intermediate size features is best near older, more stable regions and is worst near coronal condensations. It is tentatively suggested that the technique might be improved by depressing the source surface above active regions, thus reducing the spherical symmetry of the model. This prediction may not be as good as that performed in 1968[6,7], but perhaps more has been learned concerning the influence of activity upon coronal structure.

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Prediction and Observation of the Coronal Structure for the Solar Eclipse of March 7, 1970

ON March 3, Schatten drew up a prediction for the coronal structure during the March 7 eclipse of the Sun[1], based on photospheric magnetic field measurements during the weeks just preceding the eclipse. I observed the eclipse under excellent weather conditions in Mexico, using cameras of 50, 120, 150 and 800 cm focal length. The pictures taken by the long focus camera show the fine structure of the inner corona, whereas the pictures with the shorter focus cameras reveal the streamer structure of the outer corona.

Table 1 compares the predicted and observed features. Position angles are measured counter-clockwise from the north pole.

Table 1. PREDICTED AND OBSERVED FEATURES IN THE CORONA OF MARCH 7, 1970

Prediction		Observation	
30°–70°	Helmet streamer, centred on 50°	30°–70°	Helmet streamer, centred on 45°; the most conspicuous feature of the corona
75°	Coronal arches		Missing
95°	Bright feature (condensation)	88°–116°	Bright region with coronal arches, superimposed on long streamer
100°	Coronal arches		
110°	Coronal arches		Missing
100°–120°	Streamer	100°–120°	Long streamer
120°–150°	Broad streamer without arches, inclined to the equator	126°–148°	Narrow streamer, inclined to the pole
145°	Narrow streamer		
235°	Narrow streamer		Missing
235°–270°	Broad streamer without arches		Missing
278°	Coronal arches		Missing
288°	Bright feature (condensation)		Bright region with arch-like structure
292°	Coronal arches		
285°–310°	Helmet streamer, centred on 302°	280°–315°	Broad (helmet ?) streamer

The most remarkable structure of the corona is the long helmet streamer in the NE-quadrant, which is connected with a large filament and is correctly predicted. The predictions for the intense and long double streamer in the SE-quadrant and for the helmet streamer around 300° are reasonably good. In the innermost corona the brightest regions are found over the activity zones, at 70°, 105°, 260° and 295°. The streamers in Schatten's drawing are radial or inclined to the equator, whereas the actual streamers have a preferential inclination toward the polar axis. The most serious discrepancy between prediction and observation is found in the SW-quadrant. Here the corona shows its lowest intensity—even lower than around the poles—and the predicted long streamer is completely absent. Coronal arches are predicted in all but the SW-quadrant. In fact, arch-like structures are found in all quadrants and the most pronounced of them just in the SW-quadrant at 250°.

According to Schatten the corona should be more elliptical in appearance than the corona of September 22, 1968. In fact, the ellipticity has diminished during recent years. At the representative distance of two solar radii from the centre the ellipticity amounted to 0·16 in 1966, to 0·06 in 1968 (ref. 2) and to 0·01 for the March 7 eclipse.

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Solar UV Flash Spectrum 1400 Å–1960 Å

FIVE spectroheliographs were flown on an Aerobee 170 rocket from Wallops Island into the total solar eclipse to photograph and scan photoelectrically the ultraviolet and XUV flash spectrum of the Sun.

The five instruments were as follows: two Wadsworth mounting spectroheliographs with single gratings ($f=50$ cm, 2,400 lines mm^{-1}, linear dispersion 8·3 Å mm^{-1}), one with a wavelength range 150–435 Å (*a*), the other covering 700 to 1060 Å (*b*); (*c*) a modified Wadsworth mounting spectroheliograph designed to photograph the profile of the Lyman α line during the flash phase (Fig. 1); a plane grating (2,400 lines mm^{-1}, fifth order at 1216 Å) provided the high dispersion, followed by a Wadsworth mounted concave grating acting as the image forming element and "order sorter" ($f=50$ cm, 600 lines mm^{-1}, linear dispersion at Lyman-α 1·14 Å mm^{-1}); (*d*) a folded Wadsworth mounting spectroheliograph with single grating (Fig. 2, $f=50$ cm, 2,000 lines mm^{-1}, linear dispersion 9·75 Å mm^{-1}, wavelengths 1400–1960 Å); the folding mirror is introduced to reduce stray light before second contact in order to record the ultraviolet continuum in this wavelength interval; (*e*) a folded Wadsworth mounting spectroheliograph, with four scanning channels, covering 1300–2000 Å (Fig. 3), $f=110$ cm, 1,200 lines mm^{-1}).

There were three steps in the process by which the rocket was pointed at the Sun. First, coarse pointing was provided by an Aerojet Mark II tri-axial pointing system. Fine pointing, necessary because of the narrow field of view of the spectroheliographs, was accomplished by a solar eclipse sensor developed by NRL together with NASA Ames Research Center and Exotech Inc., Rockville, Maryland. An automatic gain control system provided a constant angular error signal independent of the rapidly changing intensity of the diminishing crescent, over the interval 1×10^{-2} solar constants to 2×10^{-4} solar constants. The telemetered value of the intensity between 6800 and 10700 Å was used to indicate the moment of second contact. After coarse acquisition of the Sun the sensor controlled the jet valves until the crescent was 5 arc s wide. Then the control of the valves was passed to rate integrating gyros (Honeywell GG87), which kept the payload pointing at the Sun within ±2·5 arc s, with a slewing rate of 2 arc s per second.

An electronic timer was started by the solar sensors when the width of the crescent was 5 arc s. Three different sequences were programmed for each camera which could be changed after lift-off by command from the ground in case of extreme deviations of the trajectory, which would have meant different scanning rates and different durations of totality.

The maximum apogee was 160 km, the deviation of the quadrant elevation from the calculated value was less than 0·6°, and the azimuth angle was within 6° of the predicted value. The roll angle of the rocket was decisive, because of the alignment of the crescent with the dispersion of the spectroheliographs, and was maintained within ±1° of the ideal value. The scanning speed of the Moon's limb over the edge of the Sun at second contact was about 0·42 arc s per second, and the duration of totality was 109 s.

The photographic instruments worked as planned, but the photoelectric instrument did not scan the spectrum

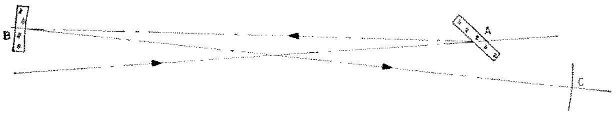

Fig. 1. The Lyman-α profile spectroheliograph. A, Plane grating; B, concave grating which forms an image on the film plane C.

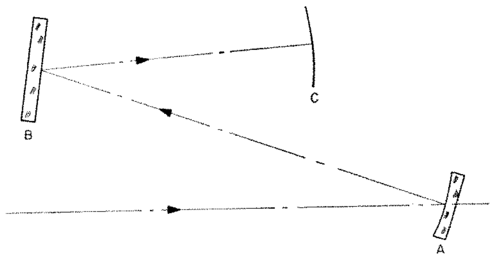

Fig. 2. Middle ultraviolet spectroheliograph. A, Concave grating, Al+MgF coating; B, plane mirror, Ge+ZnS coating; C, film plane.

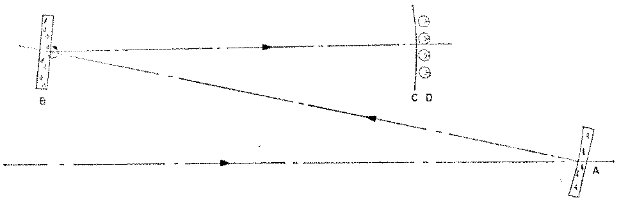

Fig. 3. Four-channel scanning spectrometer. A, Concave grating; B, plane mirror oscillating ±85° to sweep the spectrum over the exit slits; C, focal plane with four curved exit slits; D, four ultraviolet multipliers.

because of a malfunction of the motor which was supposed to drive the scanning mirror. Only limb darkening curves at discrete wavelengths could be obtained. Because of a failure in the recovery system, the payload sank into 1,770 m deep water in the Atlantic, 112 km from the Virginia coast. It was located and recovered on March 22 using CURV III (Cable-Controlled Undersea Research Vehicle) operated by the Naval Undersea Research and Development Center. Only the film strips of the middle ultraviolet instrument survived, because a cassette

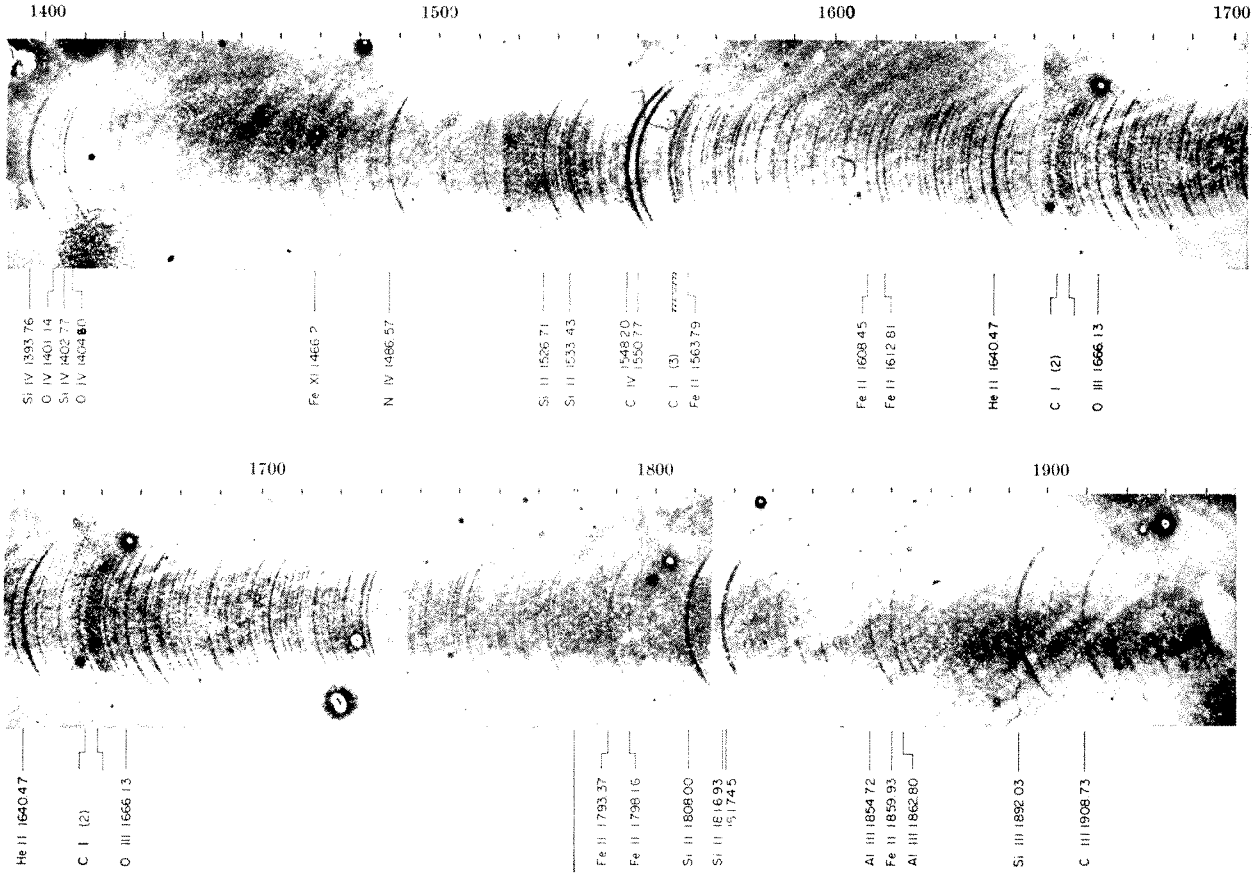

Fig. 4. Exposure No. 1. This is a five second exposure before second contact in which the Moon's limb occults the photosphere from −4·5 arc s to −2·2 arc s (the position of the Moon's edge relative to the solar limb, obtained from radar observations of the rocket trajectory).

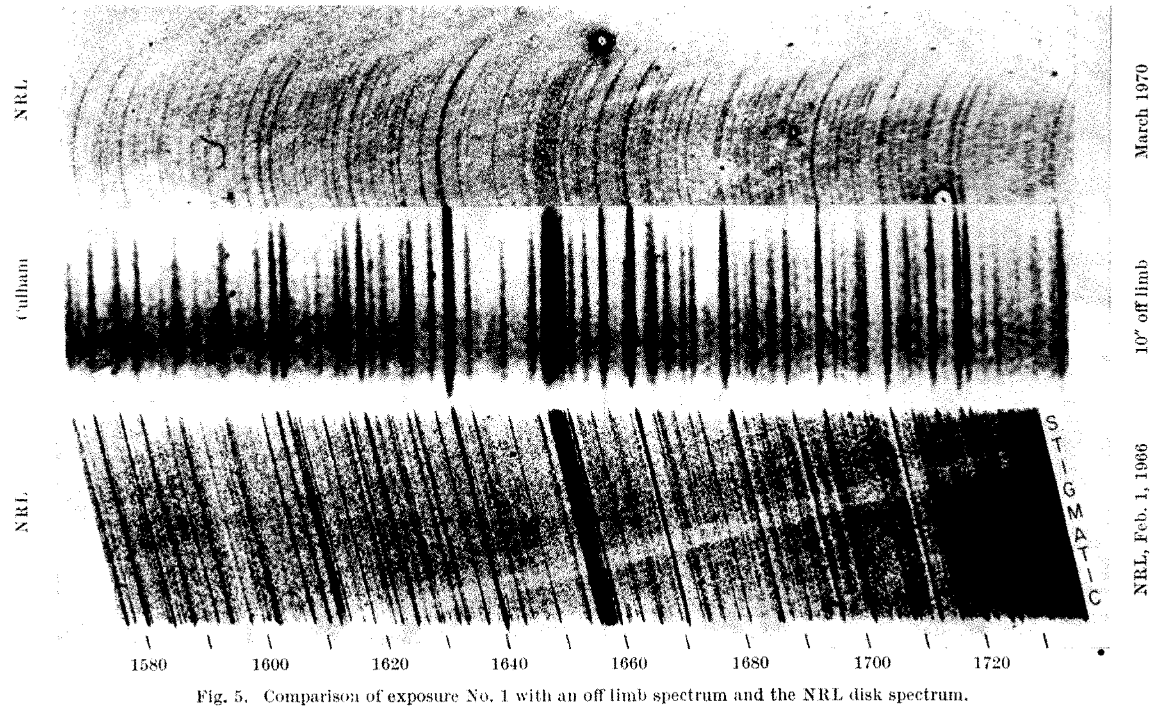

Fig. 5. Comparison of exposure No. 1 with an off limb spectrum and the NRL disk spectrum.

surrounding the film drum was coated with teflon, which slowed down the electrolysis in the salt water between the film and the aluminium of the cassette. The films were developed in straight Kodak D19 developer for 80 s at 2° C.

Fig. 4 shows spectrum No. 1, which represents the combined outer limb photospheric spectrum, the chromospheric spectrum and coronal spectrum. The photospheric continuum above 1800 Å is visible, but the background fogging makes any quantitative measurement impossible. The silicon continuum below 1525 Å can be seen.

Although a complete identification list cannot be given yet, some characteristic features of the identified lines can be discussed.

(*a*) Lines from neutral elements: (1) Many Si I lines between 1543 Å and 1565 Å have been identified by comparison with wavelength measurements in an arc spectrum by Radziemski and Andrew[1] (Table 1). All Si I lines show a crescent length which is slightly longer than the crescent of the photospheric continuum. Therefore they originate in the very low chromosphere. Fig. 6, which shows a sequence of spectra before and after second contact, verifies this. The Si I lines have all disappeared in the 1·6 s exposure obtained shortly after second contact, but they are still visible in the 1·6 s exposure shortly before second contact. All other lines remain. (2) In the wavelength range 1560 Å to 1660 Å the C I lines in Table 2 have been identified. The appearance of the C I lines is quite different from that of the Si I lines. Their crescent is longer, nearly equal to that of the Fe II lines. (This can be seen by comparison of the C I lines at 1561 Å and the nearby Fe II lines at 1558·71 Å and 1559·10 Å.) The time sequence spectra of Fig. 6 substantiate this. All Si I lines disappear shortly after second contact, but the C I lines are still there with only slightly changed intensity (see Fig. 6, spectra 3 and 6). The C I lines also show quite different behaviour to other lines in their disk to limb intensity change. This can be seen in Fig. 6 (where the solar disk spectrum was obtained by Purcell and Tousey[3]) comparing the Fe II lines at 1608·45 Å, 1610·93 Å and 1612·81 Å and the nearby C I lines at 1613·4 Å, 1613·9 Å and 1614·7 Å. The disk and the eclipse spectra show approximately the same density for the Fe II lines, while the CI lines are barely visible in the eclipse spectrum, but are clear in the disk spectrum. (The appearance of the 1658 Å C I multiplet in the disk and the limb spectrum confirms this.) Furthermore, the prominence shows very clearly in C I (and He II) but diffuse in more highly ionized lines. It is therefore very likely that the C I lines represent the spicules. The fact that the spicules shield each other when seen at the limb would explain this behaviour of the C I lines. (The amount of spicule surface seen at the limb is approximately the same as seen on the disk.)

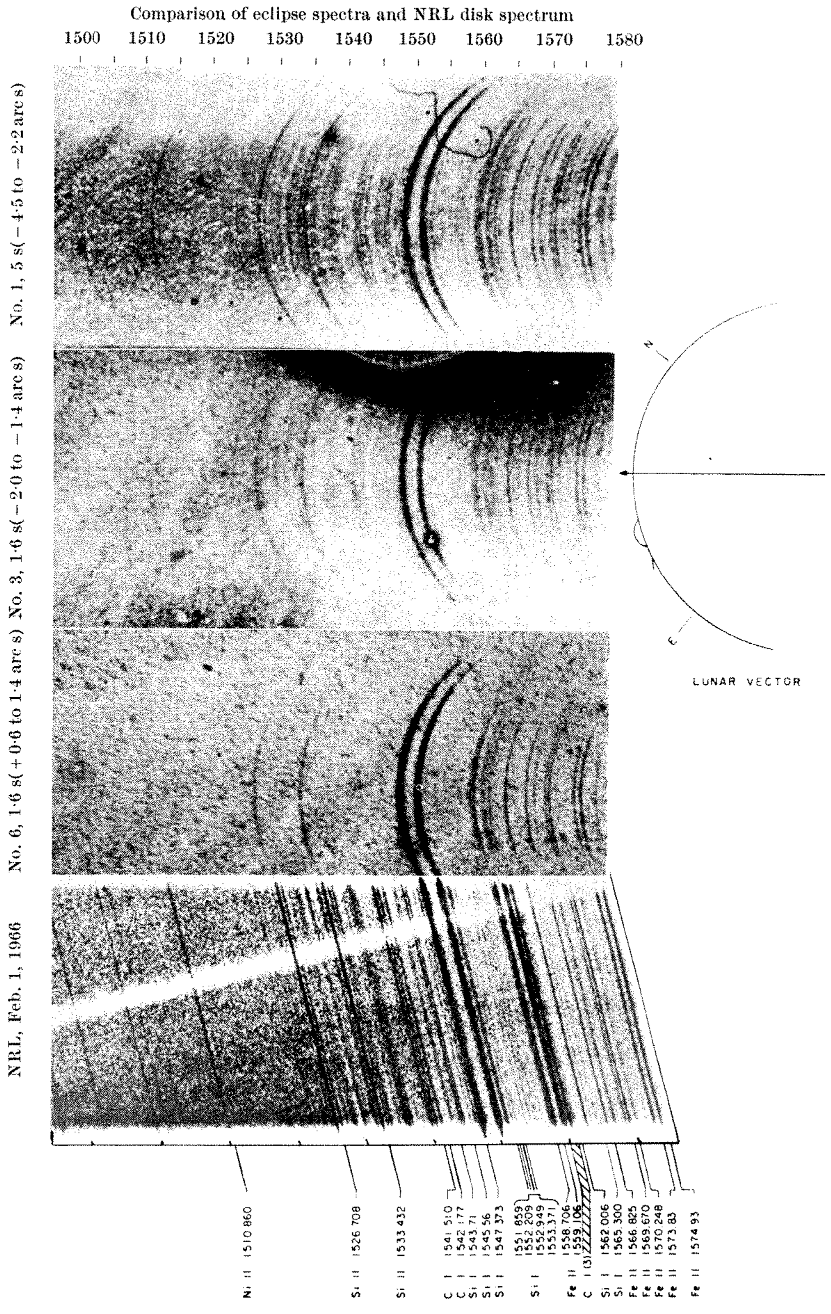

Fig. 6. A sequence of spectra before and after second contact. For exposure No. 1, see caption to Fig. 4. No. 3 was exposed to 1·6 s just before second contact when the coverage of the Sun by the lunar limb was −2 to −1·4 arc s. No. 6 is a 1·6 s exposure just after second contact when the coverage was +0·6 to +1·4 arc s.

Table 1. SI LINES

λ (Å) Eclipse	λ (Å) Laboratory	Configuration	Term	J–J
1543·74	1543·713	?		
1545·13	1545·095	?		
1545·66	1545·565	$3s^2\,3p^2-3s^2\,3p\,10d$	$g^3\,P-{}^3D^0$	2–3
1546·64	1546·559	$3s^2\,3p^2-3s^2\,3p\,10d$	$g^3\,P-{}^3P^0$	2–2
1547·49	1547·373	?		
1551·78	1551·8595	$3s^2\,3p^2-3s^2\,3p\,9d$	$g^3\,P-{}^3D^0$	2–3
1552·18	1552·2089	$3s^2\,3p^2-3s^2\,3p\,9d$	$g^3\,P-{}^3D^0$	2–2
1552·73	1552·9498	$3s^2\,3p^2-3s^2\,3p\,9d$	$g^3\,P-{}^3P^0$	2–2
1553·36	1553·3712	?		
1554·71	1554·7021	?		
1555·45	1555·5157	$3s^2\,3p^2-3s^2\,3p\,10s$	$g^3\,P-(3/2,\,1/2)^0$	1–2
1556·16	1556·1626	?		
1556·48	1556·5273	$3s^2\,3p^2-3s^2\,3p\,8d$	$g^3\,P-{}^3P^0$	1–0
1561·99	1562·0065	$3s^2\,3p^2-3s^2\,3p\,8d$	$g^3\,P-{}^3P^0$	2–2
1565·30	1565·320*	$3s^2\,3p^2-3s^2\,3p\,9s$	$g^3\,P-{}^1P^0$	0–1

* C. Moore, 1967.

(*b*) Lines from one time ionized elements: (1) He II–1640·47 Å (from ref. 4 or unpublished work of Kelly)—shows remarkable limb brightening. This line seems to extend far out into the upper chromosphere, and it can still be seen when the Moon covers 7 arc s of the chromosphere. The prominence is clearly visible in He II. (2)

Table 2. CI LINES

λ (Å) Eclipse	λ (Å) Laboratory[1]	Transition	Multiplet
1561·4	1561·4382	$2\,p^3\,P_2$–$2\,p^1\,{}^3D_3$	3
	1561·3668	${}^3P_2$–${}^3D_1$	3
	1561·3400	${}^3P_2$–${}^3D_2$	3
1560·6	1560·7088	${}^3P_1$–${}^3D_1$	3
	1560·6820	${}^3P_1$–${}^3D_2$	3
1560·2	1560·3095	${}^3P_0$–${}^3D_1$	3
1614·7	1614·5068	$2\,p^3\,P_2$–$3\,s\,{}^1P_1$	
1613·9	1613·8033	${}^3P_1$–${}^1P_1$	
1613·4	1613–3763	${}^3P_0$–${}^1P_1$	
1658·25	1658·1210	$2\,p^3\,P_2$–$3\,s\,{}^3P_1$	2
1657·80	1657·9066	${}^3P_1$–${}^3P_0$	2
1657·30	1657·3790	${}^3P_1$–${}^3P_1$	2
1656·98	1657·0080	${}^3P_2$–${}^3P_2$	2
1656·81	1656·9286	${}^3P_0$–${}^3P_1$	2
1656·24	1656·2670	${}^3P_1$–${}^3P_2$	2

Al II (1670·79 Å) is very similar to the He II line. This identification seems to be justified in view of the crescent length, which is much longer than that of the Fe II lines. Burton *et al.*[5] identified this line as Fe II, blended with Al II. (3) The Si II lines (1526·71 Å, 1533·43 Å, 1808·01 Å, 1816·93 Å and 1817·45 Å) also extend into the upper chromosphere. The strong, longer wavelength lines at 1808 Å, 1816 Å and 1817 Å can be seen further than 7 arc s. (4) The numerous Fe II lines are considerably weaker than the Si II lines. They disappear between 4·9 and 5·7 arc s into the chromosphere.

(*c*) Lines from two times ionized elements: (1) C III (1908·73 Å) (ref. 5), O III (1666·13 Å, $2p^2\ {}^3P_2$–$2p^3\ {}^5S_2$) (ref. 5), Al III (1854·72 Å), (1862·80 Å) and Si III (1892·03 Å) belong to the strongest of the spectrum, but their crescent length is smaller than that of the He II line. The limb-brightening of the O III line (1666·13 Å) is remarkable (see Fig. 5). The appearance of the prominence is very weak in all these lines.

(*d*) Lines from three times ionized elements: (1) These are C IV (1548·20 Å and 1550·77 Å), O IV (1401·14 Å and 1404·80 Å) (ref. 5) and Si IV (1393·76 Å and 1402·77 Å). The strongest lines in the available spectrum are from C IV. We estimate that these lines extend up to more than 17 arc s into the corona.

(*e*) Highly ionized elements (coronal lines): We can see three lines which are easily identified as coronal lines because of their crescent length, approximately 180° on spectrum 1. The lines are weak and diffuse. The quality of the spectrum in this region is poor. The wavelengths of these lines are 1425 Å ± 2 Å, 1448 Å ± 2 Å and 1467 Å ± 1 Å. Burton *et al.*[5] identified a line in the off limb spectrum at 1445·89 Å as the forbidden transition of Fe XI ($3p^4\ {}^3P_1$–$3p^4\ {}^1S_0$). The line at 1467 Å is the strongest of the three lines mentioned. All three lines are within the predicted wavelength range.

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Rocket Observations of the Corona on March 7, 1970

On several occasions we have made non-eclipse photographs of the outer corona between 3 and 9 solar radii, using a rocket-borne coronagraph[1]. The instrument consists of two small Lyot coronagraphs placed side by side. Solar occultation for each is by a circular disk placed 76 cm in front of the 25 mm objective aperture. The disks for the two instruments are mounted on a common spar, on posts which have different spatial orientations, so that the coronal sector obscured by one post is not obscured by the other. Kodak I–D emulsion is used to give maximum photographic speed for coronal light with an acceptable granularity. Exposures of approximately 3 s are sufficient, and minimize the effect of the rocket precession, which results in a slow rotation of the focal plane of the coronagraphs.

In a separate compartment there is an extreme ultraviolet (XUV) heliograph[2]. This consists of an off-axis, normal incidence paraboloidal mirror of 25 cm focal length, which forms a direct solar image on Schumann-type SC–7 film. Aluminium film filters and a platinized mirror restrict the passband to 170–500 Å.

This letter describes the records from an Aerobee 150 rocket launched at 1930 UT on March 7, 1970, from White Sands Missile Range, New Mexico, approximately two hours after the total solar eclipse in Mexico. In particular, we wished to photograph the white light corona beyond 3 solar radii, where the corona in Earth-based photographs tends to fade into the background sky when atmospheric conditions are unfavourable. The flight could not be made simultaneous with the eclipse in Mexico because the Sun was partially eclipsed at the launch site at this time, and the full solar disk was required to make the solar pointing control of the rocket work properly.

Fig. 1 shows the coronagraph record from the flight, combined with a ground-based eclipse photograph to the proper scale, kindly supplied by G. Newkirk, jun.,

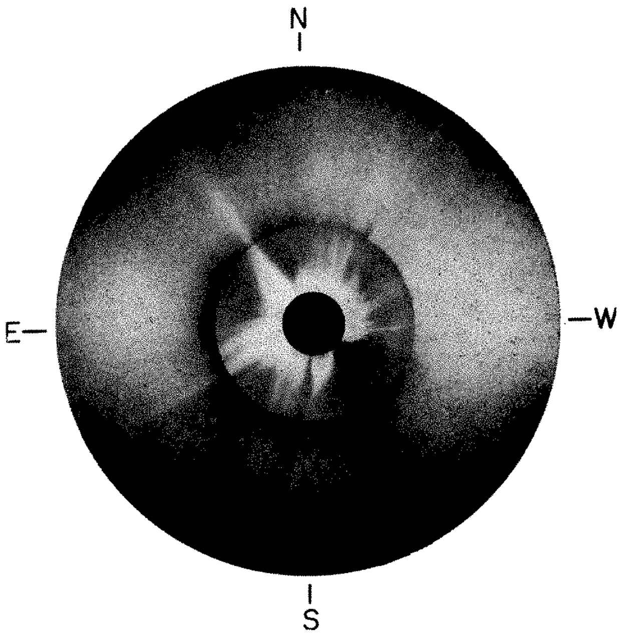

Fig. 1. White-light corona from the solar limb to 9 solar radii on March 7, 1970. The outer corona from 3 to 9 $R_\odot$ is from a pair of exposures made with the NRL coronagraph at 1930 UT. The inner corona was photographed in Mexico by G. A. Newkirk, jun., at approximately 1740 UT. In both the radial decrease in brightness has been compensated instrumentally to give a uniform exposure over the field.

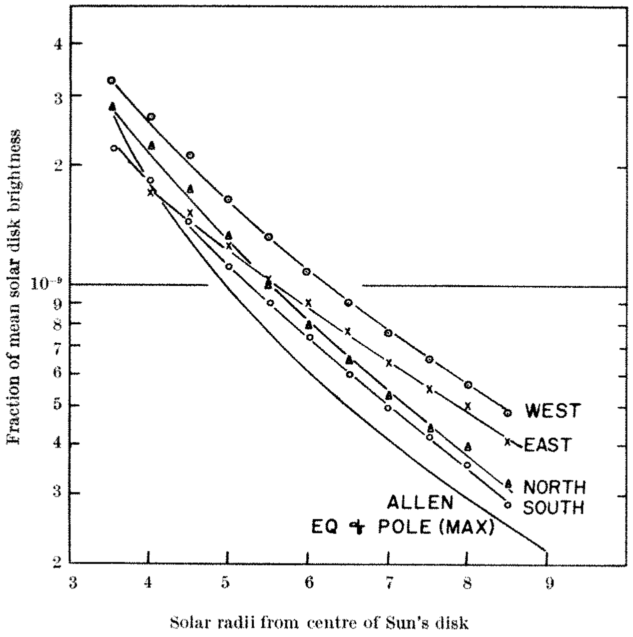

Fig. 2. Brightness of the K+F corona at the equator and poles on March 7, 1970, compared with data for solar maximum published by Allen.

director of the High Altitude Observatory of the University of Colorado. The field brightness in this composite appears nearly uniform because of the vignetting produced by the external occulting disk; the vignetting function compensates for the radial decrease in brightness of the corona. The dark arc to the east of the centre is the Moon silhouetted against the corona. The Newkirk photograph has better resolution than the rocket photograph, but is similar in that it was made with a filter which compensates for the radial decrease in coronal brightness. Our comments will be restricted to the outer picture taken with the rocket coronagraph.

The coronal streamers appear here as parallel or slightly diverging, with no detectable curvature. Therefore the range beyond 3 solar radii may not be greatly influenced by the complex magnetic fields nearer the limb. We call attention to the dark sector in the south-east, bounded sharply on one side by the strong south-east streamer, and more faintly on the other by a broad feature. The effect is probably produced by the projection of several different streamers on to the plane of the sky, but perhaps may indicate some peculiarity of the large scale magnetic field.

The rocket photograph does not show to any extent the dark sector in the south-west which is so strikingly evident in the Newkirk eclipse photograph. The darkening seems mostly associated with the region close to the limb. All streamers appear to lose contrast and disappear into the general coronal background as they approach the edge of the field at 9 radii. It is believed that the background is true corona rather than stray light.

Although it is difficult to estimate the contribution of instrumental stray light to the general background, the black appearance of the Moon suggests that stray light is negligibly small. The lunar image is at fog level in the original negatives. In this connexion it is interesting to note that sky conditions in Mexico were exceptionally good, and that streamers were recorded to elongations greater than the 9 $R_\odot$ limit of our coronagraph (private communication from Newkirk and Pasachoff). This is surprising if the stray light in our instrument is indeed small. Perhaps the ground-based films could detect a smaller difference in contrast than our film, but this was at least partly compensated by the contrast-reducing effect of residual skylight. Overall we would expect our film, which was developed to a gamma of 2·0, to have a contrast rendition comparing favourably with that of ground-based films.

Our photograph shows a prominent equatorial enhancement of the background corona. Again this seems real, with a negligible contribution from stray light as shown by the blackness of the lunar silhouette which lies in the equatorial plane, and by the fact that the original negatives showed the enhancement to be fixed in space during successive exposures while the coronagraph focal plane rotated some 60° due to the rocket motion.

The photographic advantages of the vignetted coronal image are ultimately neutralized by the increased care required in the photometry of the photograph, because film response is a strong function of the radial position in the field, and accurate measurements of position as well as density must be made on the 35 mm negative. Fig. 2 is a preliminary measurement of the equatorial and polar brightness. The data have been smoothed to some extent but include the local effects of the streamers. The curves show the equatorial enhancement, as well as the enhancement between 3 and 6 solar radii in the polar regions produced by the presence of streamers. The curve of brightness for equator and pole during solar maximum, as published by Allen[3], is included for comparison.

The two-hour difference between Newkirk's photograph and the rocket record has produced no obvious mismatch of streamers, but it is possible that small differences between our and other eclipse data may be found. On our negatives, the angle between the strong north-east and south-east streamers, projected back to their crossing point, is 82·7°. Other streamers are more diffuse and cannot be located with accuracy. We call attention to the group of three which lies to the west of north. The westernmost member of this group is 57·6° from the strong north-east streamer.

Fig. 3 shows successively longer exposures of the solar disk image, obtained with the rocket XUV heliograph. The most striking features are the corona-like emission above the limb and a narrow limb-brightened ring around the entire disk.

Purcell *et al.*[2] concluded on the basis of earlier spectroheliograms that the narrow limb was caused chiefly by Ne VII (465 Å), Mg IX (369 Å), and the progression Fe VIII at least to XI and perhaps to Fe XIV. The XUV corona, projected beyond the limb, was contributed to by all these lines, but was produced especially by the very high-stage ions of Si, Mg, and Fe, to stage XVI. Thomson scattering of solar disk radiation was ruled out as a possible mechanism because the emission was far too intense relative to the disk. It therefore seemed likely that the radiation was produced by collisional excitation of the ions by free electrons, especially in the low corona where the electron density is high. This mechanism makes the intensity proportional to the square of the electron density and will produce a steep radial gradient in the XUV corona. The gradient was actually observed to be shallow, and there was no structure corresponding to white light streamers, even in the outer XUV corona which was recorded beyond 2 $R_\odot$ in the long-exposure heliograms. It was therefore proposed that this corona was caused by resonance as well as collisional excitation, and that the unexpectedly great intensity was associated with a high abundance of Fe ions in the corona.

Whatever the relative importance of the emission mechanisms, the low XUV corona observed on March 7, 1970, has a considerable resemblance to the low white-light corona photographed on the same date. This is shown in Fig. 4, where one of the XUV images of Fig. 3 has been masked with a disk of black paper to show only the features projected beyond the limb. This is compared with a short-exposure eclipse photograph from the US east coast one hour earlier. One difference is that the XUV corona has depressions not seen in the white light corona.

We have noticed in several previous XUV disk photographs that limb brightening is suppressed in the neighbourhood of Hα limb prominences. In Fig. 4 the depressions also seem to occur near the positions of Hα limb features.

Although the long-exposure heliograms of Fig. 3 do not reveal any structure which may be imagined to correspond to a white light streamer, we do not consider that the absence of XUV streamers has been adequately proved. Our long-exposure heliograms have all suffered to some

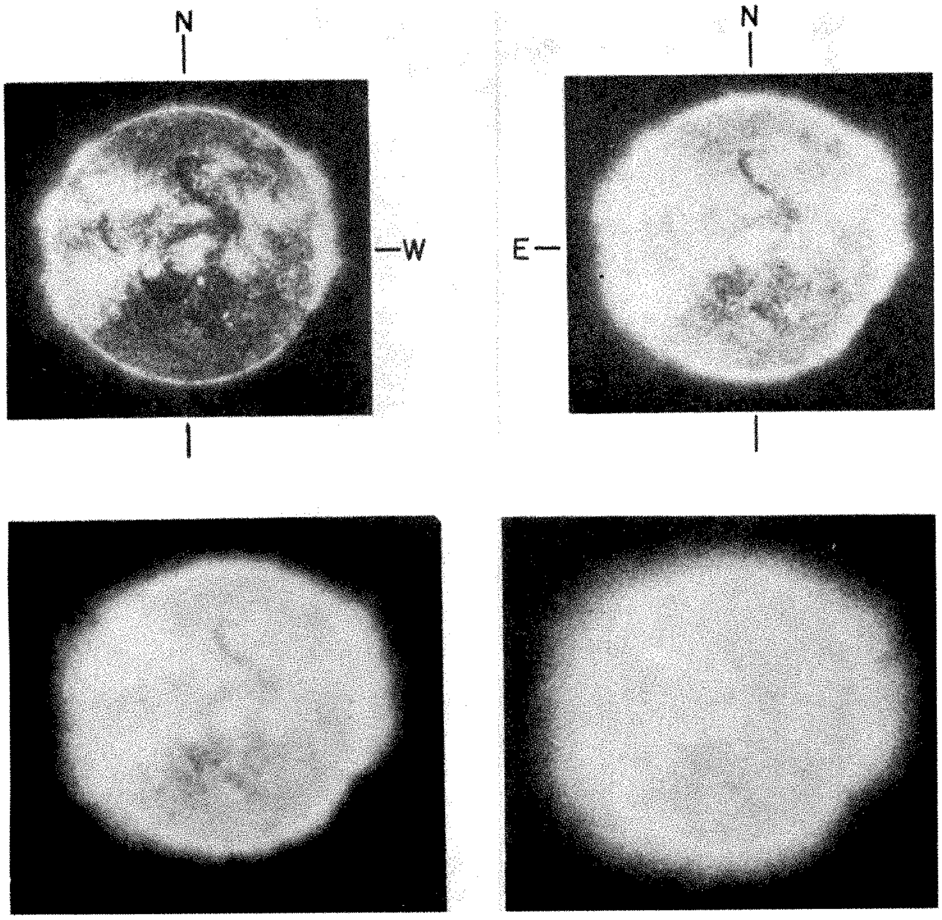

Fig. 3. The solar disk in the band 170–500 Å photographed with successively longer exposures to record the corona. March 7, 1970, 1930 UT.

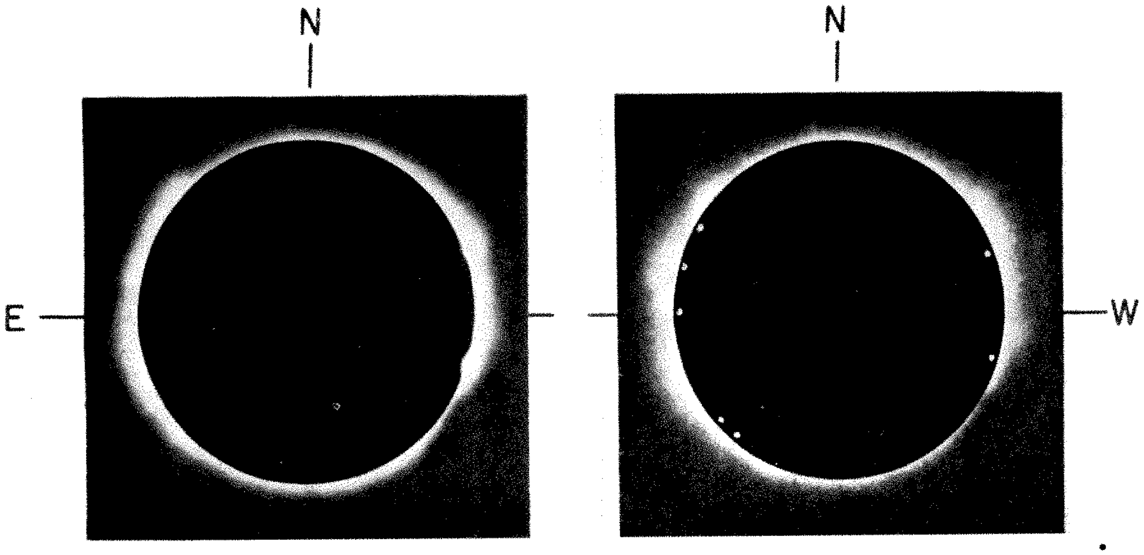

Fig. 4. A short-exposure white light eclipse photograph (left) taken at 1835 UT, compared with an XUV heliogram from Fig. 3. The heliogram is masked to show only the emission beyond the limb. Approximate positions of Hα limb features are indicated on the mask by white dots.

extent from image rotation produced by rocket precession. This may have masked any streamers. We hope eventually to make long exposures in a three-axis stabilized system free of image rotation. It is desirable also to have the Moon in the field of view as in Fig. 1 as an indicator of instrumental stray light.

We thank the National Aeronautics and Space Administration which has supported this and many previous rocket coronagraph experiments. Mr Robert Krupinski of this laboratory made the short-exposure eclipse photograph in Fig. 4. We also thank Mr R. T. Seal, Mr C. B. Snyder, and Mr R. K. Chaimson for help. The coronagraph optical system was designed and manufactured by Tropel Inc., and the rocket pointing control was manufactured and operated by the University of Colorado.

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The Lockheed-Aerospace Eclipse Expedition

We used highly portable equipment which could be set up on almost any surface, and left the decision on the location of the observing site as late as possible so as to take advantage of the latest weather forecasts. Our range of possible sites included all points along the path of totality between Tallahasee, Florida, and Norfolk, Virginia. We flew into Atlanta, Georgia, about 32 hours before totality, and a weather assessment there encouraged us to fly to Norfolk where a final decision was made to motor south-west into North Carolina. The final site selection was made on the morning of the eclipse, and we observed from a farm near Beargrass.

Four coronal photographic experiments were carried out. The first, conceived by H. E. Ramsey of Lockheed, used an $f/12$ plane polarizing telescope of 24-inch focal length to observe the coronal green line at 5303 Å. The filter loaned by Spectrolab had a passband of 1 Å at 5303 Å, and we used Kodak 103 AG film. Exposures were made at four polarizing positions 45° apart, and a fifth (off band) exposure was made in the nearby blue continuum by tilting the filter. Fig. 1 is typical of the on-band exposures. Visual comparison of the four photographs shows no important intensity differences.

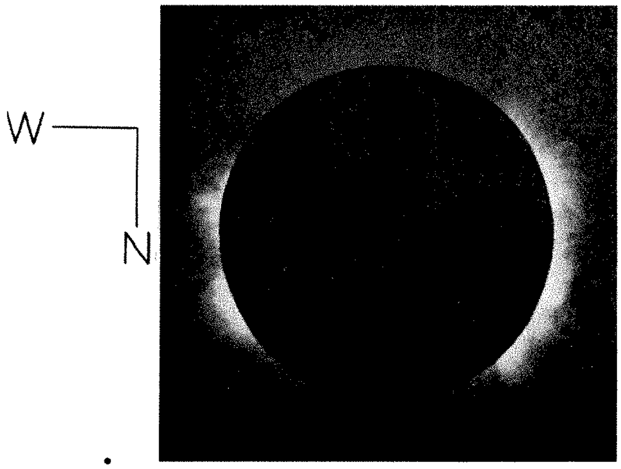

Fig. 1. The green line corona.

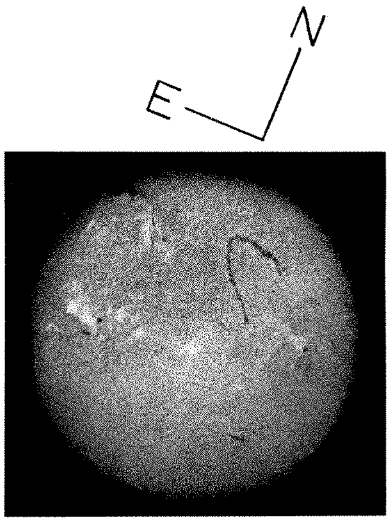

Fig. 2. The solar disk at the time of the eclipse photographed in the light of Hα (Lockheed Solar Observatory, Saugus, California).

The chief value of the green line photographs is in the study of coronal rays, arches, and other structural detail. We have found a close correspondence in the general appearance of the green line corona with the white light corona although our white light photographs do not show nearly as much structural detail. Further comparison of the green line pictures with higher resolution photographs in white light taken by Sheldon Smith of NASA Ames Research Center shows an even greater similarity in the form and structure of the corona. No structures are visible in the green line that are not also visible in Smith's white light photographs showing more extensive detail. We feel certain of the reality of the structure at 5303 Å because an exposure of equivalent length taken with the 5303 Å filter tuned approximately 2 Å into the blue wing of the line reveals only a narrow, irregular band of corona around the limb with no structural detail.

The green line structure is sparse at the poles and pronounced in the active regions. A system of closed coronal arches is visible in the NW quadrant of Fig. 1 (upper right). We have found that other systems of loops intermingled with the ray structure on the east and west limbs can be identified in the original negatives through high contrast printing.

The largest of the prominences visible during the eclipse is an extension of the large filament on the NE quadrant of the solar disk seen in Hα light in Fig. 2. The filament (or prominence) is in the northern part of the active region zone and does not seem to be associated uniquely with any particular loop system in the inner corona. In the white light photographs of Sheldon Smith, however, the outer corona reveals an extensive helmet and streamer above this prominence as well as above the prominences on the SW and NW limb. On the SW limb, the northern end of a loop prominence seems to terminate in a very tiny active region. This region began developing on the solar disk on the preceding day around 1700 UT. During the eclipse a very small flare and corresponding small surge occurred in this active region from about 1733 to 1741 UT. The active region lies just within the southern boundary of the active region zone. The green line corona above the region is not intense although the region is situated almost exactly on the limb. Apparently the green line exposure is so short that it reveals only the base of the helmet structure and streamers over this active zone. Above the small prominence on the NW limb, however, concentric arches within the helmet can be seen in the green line photographs as well as in Sheldon Smith's white light photographs.

Another camera used aero infrared Ektachrome to obtain pictures of the corona. Exposure times were from 32 s to 1/60 s with a Wratten No. 12 filter. Streamers can be seen out to nearly five solar radii on the 4 s exposure. In general, the structures agree well with those seen on the normal colour film, but the radial density gradient on the infrared film seems to be somewhat lower. Properly exposed regions, outside the white aureole, show a deepening blue with increasing radius.

A third instrument using a 680 mm focal length solid catadioptic objective (loaned by Boller and Chivens) and a camera with an automatic film transporting mechanism (loaned by EPOI West) was devoted to white light colour photography of the entire eclipse. These photographs include the diamond ring effect, Baily's beads, prominences, and exposures showing the inner corona and outer corona to a distance of 2·3 solar radii.

Broad-band Hα pictures of high contrast were taken for details of the coronal ray structure. A graduated series of exposures were taken on a four-inch Questar telescope with a 250 Å passband filter centred at 6563 Å, using Kodak SO-375 film.

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Swiss Solar Eclipse Expedition to Mexico

THE Swiss expedition was split into two groups operating at Nejapa and at Miahuatlán to carry out a programme of photometric, polarimetric and spectroscopic observations of the solar corona (Figs. 1 and 2). We also obtained motion pictures of the eclipse and colour photographs of the corona for educational purposes.

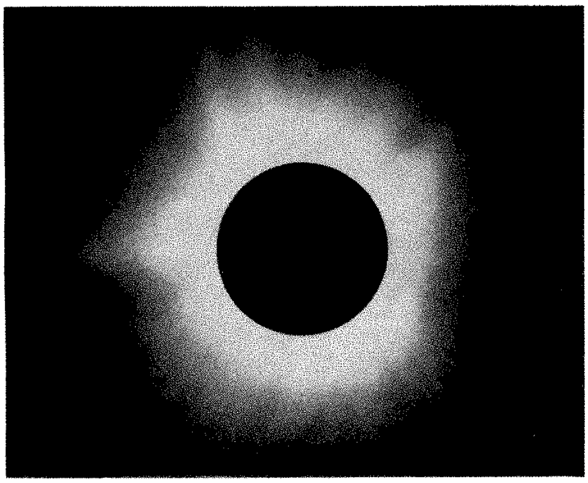

Fig. 1. The streamers of the outer corona. The axis runs from upper right (south) to lower left. The picture was taken using a one-mirror coelostat, so east is at upper left.

For more than thirty years I have been observing the coronal emission lines at the Arosa observatory, but the coronagraphic method is restricted to the relatively bright innermost corona. Even the brightest line, the green line at 5303 Å, can hardly be observed at more than 6′ from the limb. The excitation of this line can be by photospheric radiation as well as by electron collisions, and to separate the contributions of these two mechanisms the line intensity has to be observed to much larger distances than can be reached with coronagraphs. To do this we used a low dispersion spectrograph of high luminosity, which could be rotated about the axis of its collimator so that the radial distribution of the intensity of the green line could be measured at sixteen different position angles and out to about one solar radius from the limb. The spectrograph was built for the green line, but it gives the whole spectrum from 3900 to 6500 Å.

A thorough knowledge of the electron density is needed for the interpretation of the line intensities and their variations. This was achieved by observing the distribution of the white light of the corona, using two cameras of focal length 120 cm and 150 cm. The white light is photospheric light scattered by free electrons and the photometric measurements can easily be transformed into electron densities.

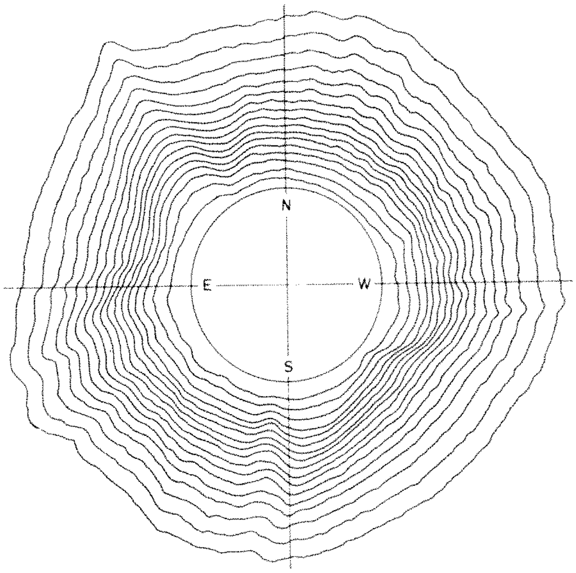

Fig. 2. Isophotes of the corona. There is no appreciable flattening. The most conspicuous feature is the long and isolated streamer in the NE-quadrant. The weakest part of the corona is found in the SW-quadrant, and a pronounced dark rift is seen near the S-pole.

The corona is not a homogeneous atmosphere, but consists of a multitude of individual streamers again built up of finer details which are visible on pictures of high resolution. We used a camera of 8 m focal length which shows only the inner parts of the corona, but it is in the innermost regions where the corona shows the finest and most fascinating structures, especially over centres of activity. These structures are formed by magnetic fields emanating from deeper layers. This is the only way to get an insight into the shape of the magnetic fields in the immediate surroundings of the Sun.

The theory of scattering predicts strong polarization of the white light of the corona. The degree of polarization depends considerably on the angle of scattering, and a streamer the axis of which is perpendicular to the line of sight shows stronger polarization than one with a smaller inclination. It is only by polarimetric measurements that the inclination of the streamers to the line of sight can be determined, and an extensive programme of

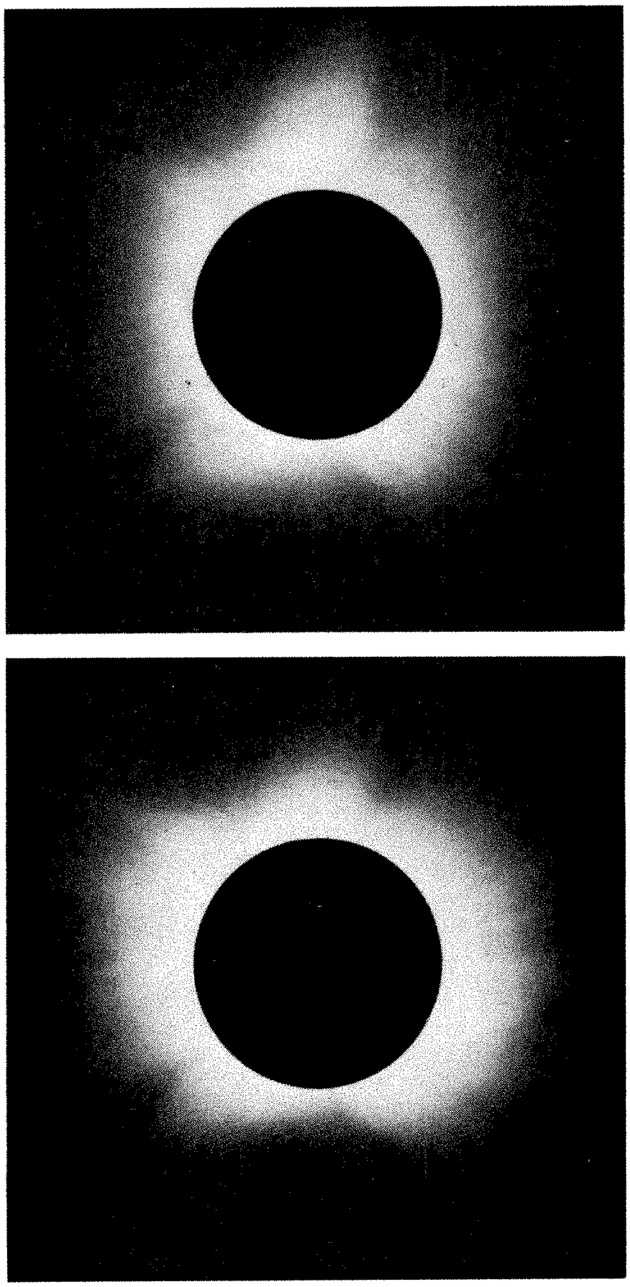

Fig. 3. Two pictures of the corona in linearly polarized light. In the upper picture the direction of polarization is vertical, in the lower picture horizontal. The axis runs from upper right (north) to lower left (south). East is at upper left.

polarimetric measurements was carried out using a camera of 120 cm focal length (Fig. 3).

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Los Alamos Airborne Observations

THE eclipse of March 7, 1970, was observed off the east coast of Mexico from an Air Force NC-135A instrumented by the US Atomic Energy Commission for chromospheric, coronal, and geophysical observations.

The proper intercept flight path was calculated from Bessel elements of the eclipse from the 1970 *American Ephemeris and Nautical Almanac*. The equipment was built to view the eclipse (63° altitude) at an azimuth of 100° relative to the aircraft nose, so the no-wind aircraft path needed to be 5° to the left of the totality path for a west coast intercept and 13° to the right (53°·3 true) for the actual east coast intercept. The true altitude of the aircraft was 36,380 feet. The actual ground speed of 508 knots and ground track of heading of 53°·7 true give a duration of 5 min 39 s (using an effective Δt = ephemeris time minus universal time = 40 s). The actual duration measured was 5 min 30 s, two minutes more than the 3 min 28 s expected on the ground.

The nine seconds discrepancy between the predicted and measured durations can be due to a number of effects such as navigation errors, inaccurate Bessel elements, or to the kilometre or more uncertainty in the actual lunar radius at both the second and third contact points.

A practice mission from Kelly Air Force Base was flown on March 4 at the west coast intercept. The navigation proved to be somewhat more difficult at that location, and the proximity of the ground observers prevented the continuation of the track (with possible contrails) to the east for calibrations after the eclipse. Therefore even though totality lasted a few seconds longer at this site, the east coast intercept was chosen because of advantages of higher altitude possible at a shorter range from the base, easier navigation, and the possibility of a long post-eclipse calibration track. A second post-eclipse calibration run of ten minutes was made on the way home after last contact on both rehearsal and eclipse days.

The chromospheric and coronal observations were all made by instruments with combinations of gyro and photoelectric tracking. Hydraulic power was used to maintain the pointing of the heavier instruments instead of electric motors. Photographs with the equipment of highest resolution, a coronal camera, had pointing errors of less than 10 s of arc.

The coronal camera had an *f*/6 lens of 610 mm focal length with three cemented doublets. The electronic computing machine lens had its best focus between 5000 Å and 7000 Å where the photographs were made. The automatic control system enabled C. F. Keller to record sixty-eight photographs in various polarization planes on Kodak 2475 film with exposure times ranging from 0·01 to 10 s. The unusually spiky appearance of the active corona was recorded out to thirteen radii from the solar centre.

Lower resolution photographs were taken with an emission line camera by R. Engleman and R. E. Partridge. Nine 13-inch *f*/6 lenses produced images on a single sheet of Kodak 2475 film with a patch of Kodak high speed infrared film. The forbidden emission lines observed, with half widths of the filters between 3 and 5 Å, were: 5303 Å (Fe XIV), 5303 Å (Fe XIV) with polaroid, 6374 Å (Fe X), 5694 Å (Ca XV), 7892 Å (Fe XIII), and 5890 Å (Na I). Background photographs were taken at 6426 Å, 5357 Å, 5277 Å. Three good exposures, of 27, 27 and 105 s, were obtained. Photometry will show the structure of the corona for the various emission lines.

A Fourier transform infrared spectrometer was used by K. H. Olsen and C. R. Anderson to measure the chromospheric and coronal spectra in a circular spot of 10 min of arc at both the second and third contact points. With a resolution of $2\ \text{cm}^{-1}$ the spectrum, including the emission lines of Si X, He I, H I, and others not yet identified, was recorded with an InSb detector at wavelengths between one and three microns.

An *f*/8 telescope of eighty-inch focal length was used by M. M. Hoffman and D. H. Liebenberg to record the line shape of the green and red iron lines in the corona. Narrow band optical line filters were used in front of a Fabry–Perot interferometer, and the varying freon gas pressure gave a spectral scan of the selected emission line at every point in the coronal image. The fringe pattern was recorded with a high resolution image orthicon video camera coupled to a video tape recorder. The data will be analysed for line shapes and coronal temperatures.

The behaviour of the 5577 Å and 6300 Å forbidden neutral oxygen emission lines and the 3914 Å N_2^+ emission line from the Earth's upper atmosphere was also recorded along paths through and in the lunar umbral shadow by M. M. Robertson and J. H. Wolcott. Scans between the lunar direction and 30° from the horizon in the direction perpendicular to the umbral path were made to study excitation mechanisms for this airglow when the solar light is rapidly cut off. Recorded intensities were very close to those expected from previous airglow measurements.

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Photometric Study of the Solar Corona

ON March 7, 1970, we observed the K and F solar corona photographically at El Gramel, Oaxaca, Mexico. The technique has already been explained[1,2], but has been improved since the 1968 experiment in Siberia. By using a graded filter—the neutral radial attenuator technique—photographs were obtained in E-radial and E-tangential polarized light which allow the polarization of the fine structure in the electronic corona to be evaluated. Thus the F corona as well as structures in the K electronic corona can be subject to precise photometry on correctly exposed photographs. Overexposed parts cannot be avoided using the traditional photographic methods (Figs. 1–4).

Photographs in natural light were obtained through a window in the attenuator, to give an indication of the conditions of the observations. It seems that for practical purposes the centre of the Moon was not affected either by the atmospheric diffused light or by instrumental light.

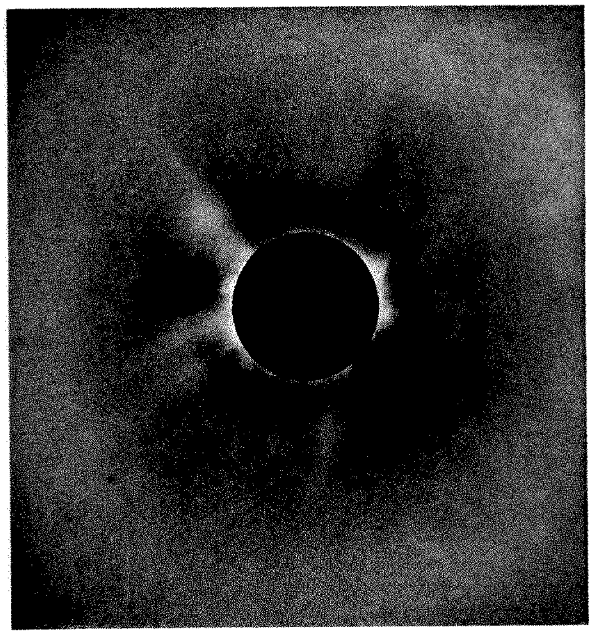

Fig. 2. The same photograph in radial polarized light.

This offers exceptional conditions for observing the solar corona. In particular, it is possible to neglect completely for $\rho > 2$ the influence of the coronal aureole, the sky brightness being the result of double diffusion[3]. It is interesting to note also that the diffusion of solar radiation by illuminated particles situated outside the shadow, and possibly between the Moon and the Earth, is not observed[4] as it is in rocket experiments.

Thus an excellent morphological study of the electronic corona is possible. The filament structure is evident and the dispersion of the intensities of the various streamers as well as their superposition shows that the slide registers a large range of streamers situated in quite distinct planes. The "dark filaments" reported in 1968 (ref. 5) were again observed (Fig. 3). It seems impossible, *a priori*, to interpret these structures as "holes" in the corona (the traditional interpretation for the coronal cavities).

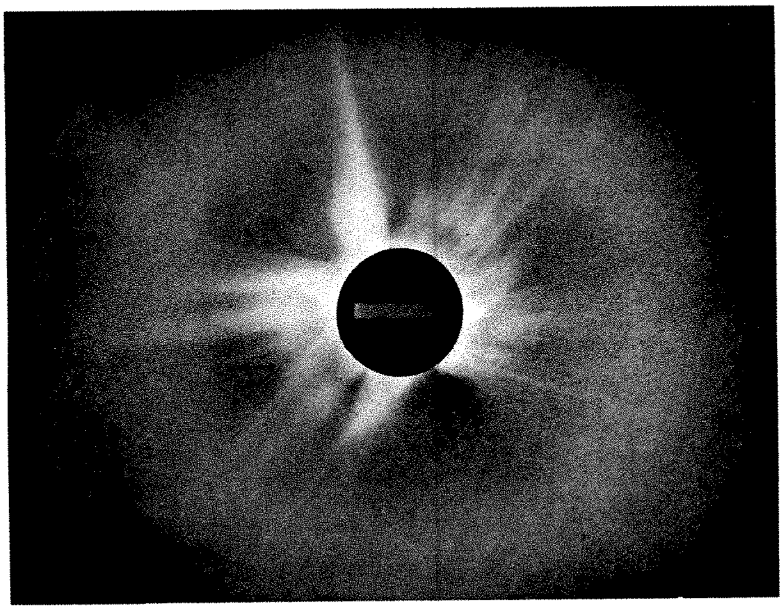

Fig. 1. Photograph of the solar corona at $\lambda = 5700 \pm 200$ Å, on March 7, 1970, 1735 UT. A rectangular window on the Moon registers the Earthshine.

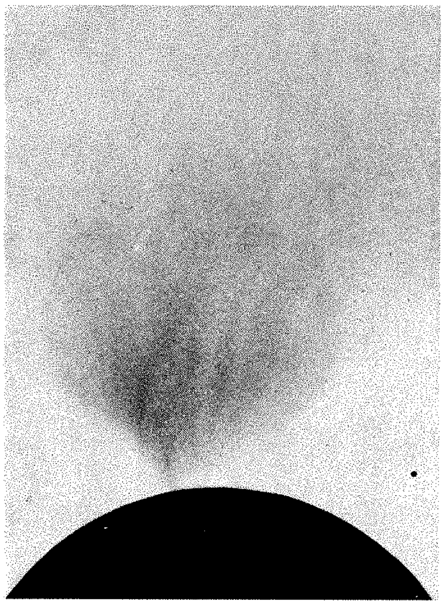

Fig. 3. Enlargement showing two "dark filaments" in the corona.

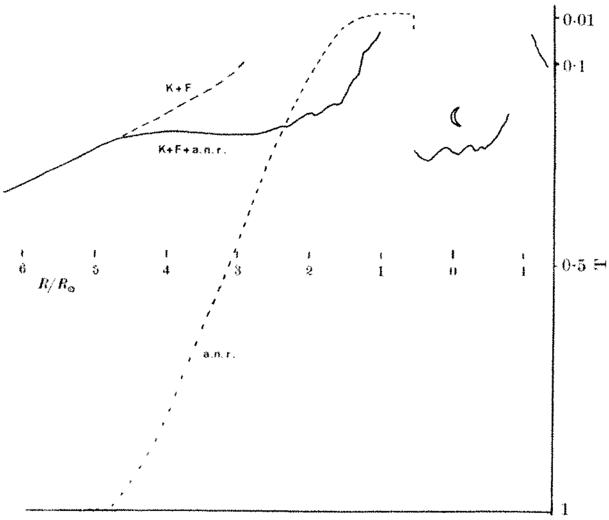

Fig. 4. Radial microphotometer tracings on the natural light half of the slide. The details of the Earthshine on the Moon are obtained quantitatively. The dashed line represents the tracing of the neutral radial attenuator.

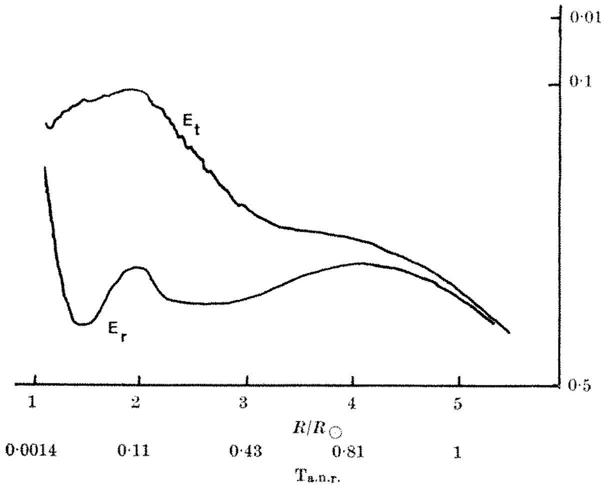

Fig. 5. Radial microphotometer tracings in radial (E_r) and tangential (E_t) polarized light, showing the variation of the polarization with $\varrho = R/R_\odot$.

The variation of the polarization rate in solar polar coordinates is given by simple photometric scanning, along the same radius, of two E-tangential and radial polarized photographs without any intermediate calculation. Two of these scans are presented in Fig. 5, which shows that the polarization rate increases rapidly from the limb to a maximum value at $\rho \sim 1{\cdot}4$ and then decreases because of the growing importance of the non-polarized F corona.

We thank Dr Michard for discussions.

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Doppler Temperature of the Solar Corona

WE have observed the thirteen-times ionized line of iron in the solar corona, Fe XIV $3p^2P_{1/2}$–$^2P_{3/2}$ at $\lambda = 5302{\cdot}86$ Å. This was done with Fabry–Perot interferometers both photographically and using a photomultiplier. The results are still preliminary, but we already have information on the Doppler temperature and motion of the corona as far out as 1·85 solar radii.

In contrast to previous observations[1], we find that the corona is in violent motion, as shown by definite spectral shifts and differences in Doppler temperature.

The photographic Fabry–Perot interferometer was placed with horizontal plates in an invar mounting which was temperature controlled to within ±1° C. The light was collected by a 10 inch diameter coelostat with a lens of focal length 145 inches. The 5303 Å line was separated with a $2\frac{1}{2} \times 2\frac{1}{2}$ inch interference filter placed near the primary focus. The light was made parallel by an auxiliary lens so that the image of the Sun and the Fabry-Perot fringes would be focused together by the camera. A K-37 aerial camera with a 12 inch lens used at *f*/7 formed the final image of the interferometer fringes and the corona together on a special Eastman Kodak 2485 high speed recording sheet film. The Fabry–Perot was equipped with Baird Atomic dielectric multilayer coatings which afforded an overall finesse of 14, yielding an instrumental half-intensity width of 0·2 Å with a plate separation of 0·05 cm.

The exposures used, 5, 15 and 60 s, were long enough to provide fringes extending to large distances from the Sun.

The photoelectric Fabry–Perot was swept with Freon-12 pressure and was also mounted in an invar case. The signal was chopped, received by a refrigerated EMI photomultiplier and was amplified by a PAR phase sensitive amplifier. The apparatus was mounted on an equatorial mount originally designed for an 8 inch reflecting telescope.

Microdensitometer tracings, made radially from the centre of the fringe system, which was at a point 0·2 solar radii from the limb on the north-west side, were first reduced to intensities using a calibration exposure developed with the plates. We corrected for the background due to the continuum by assuming an exponential decrease fitted to the intensities read between fringes. The corrected Fabry–Perot pattern thus obtained gives line shapes and wavelength shifts without complications arising from the very steep intensity gradient of the corona.

Preliminary results indicate that the half intensity width of the green coronal line during this eclipse varied between

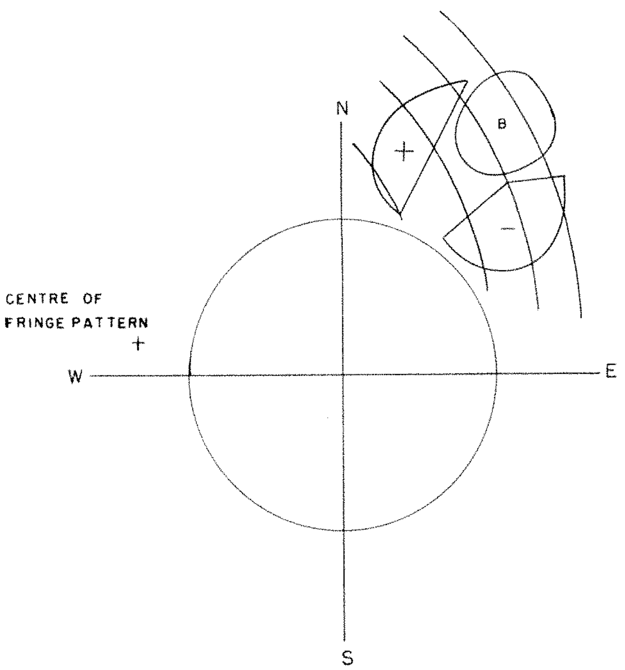

Fig. 1. Position of principal wavelength shifts and broadening of the 5303 Å line, and position of the fringe pattern. The region indicated by + has the line shifted toward longer wavelength and the region indicated by − the opposite. Region B corresponds to a decrease of intensity and a broadening of the line.

0·8 and 1·1 Å and that the intensity distribution was definitely unequal, with large intensities in the southern hemisphere and in an active area which extended up to 1·8 solar radii toward the north-east. This active area corresponds to the prominence and the strong arches and fan rays observed in this region[2], and seems of particular interest because of the large variations of width and the wavelength shifts associated with it. These variations seem to be restricted to definite localities corresponding to the arches in the white corona (Fig. 1).

The connexion between the prominences, the coronal streamers and the emission corona has long been believed to determine the structure of the corona[3]. Our preliminary results, as shown in Fig. 1, would reinforce this suggestion because they indicate a cooler fan system slowly rotating around a hotter and less dense plasma. We are studying the possibility of relating the wavelength shifts to the broadening of the line. Such a relationship could show a correlation between motion in the corona and the temperature and perhaps give a clue to the heating mechanism.

This work was supported by the National Science Foundation and by the Air Force Office of Scientific Research, USA.

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The Outer Corona at the Eclipse of March 7, 1970

Light from the vicinity of the corona at total solar eclipses may come from five possible sources: (1) the E-corona of emission lines; (2) the K-corona, a partially polarized continuum scattered by free coronal electrons; (3) the F-corona, whose Fraunhofer spectrum is usually regarded as the central part of the zodiacal light-radiation scattered by interplanetary dust; (4) superposed light from the sky, ordinarily caused by multiple scattering from distant regions in partial eclipse; (5) scattered light within the instrument superposed on the final image.

Models of the solar corona depend markedly on the evaluation of the relative contributions from sources (3), (4) and (5). The most widely accepted model of the corona is that proposed by Van de Hulst[1] and updated by Blackwell and Petford[2], for which the intensity of the F-corona exceeds that of the K-corona beyond radii, measured from the centre of the Sun, exceeding about 2·2 for sunspot maximum. Preliminary results from our expedition to Miahuatlan, Oaxaca, Mexico, indicate that the K-corona may be dominant at much greater distances from the Sun than had been thought previously.

To separate the F- and K-coronas we carried out four kinds of experiments, from which data are now being reduced. First, a new spectrograph designed by J. G. Baker registered the coronal spectrum from 3500 to 6000 Å. Second, four television cameras recording on videotape scanned the corona over a field of 5 × 7 solar diameters, with rotating polaroids and different neutral and coloured filters, an experiment designed by W. W. Salisbury and D. L. Fernald. Third, we photographed the coronal polarization. Fourth, we photographed the corona with a variety of different lenses, as in Fig. 1.

During the eclipse the sky was exceptionally clear and Rayleigh blue to the edge of the solar disk. This condition was particularly favourable for observing the corona. To the naked eye, the corona appeared stellated, with long streamers plainly visible to four or five solar diameters. These structures would ordinarily have been obscured if any haze had been present. They also showed clearly on long-exposure calibrated photographs. Two of them, located at position angles 12° and 94°, extended

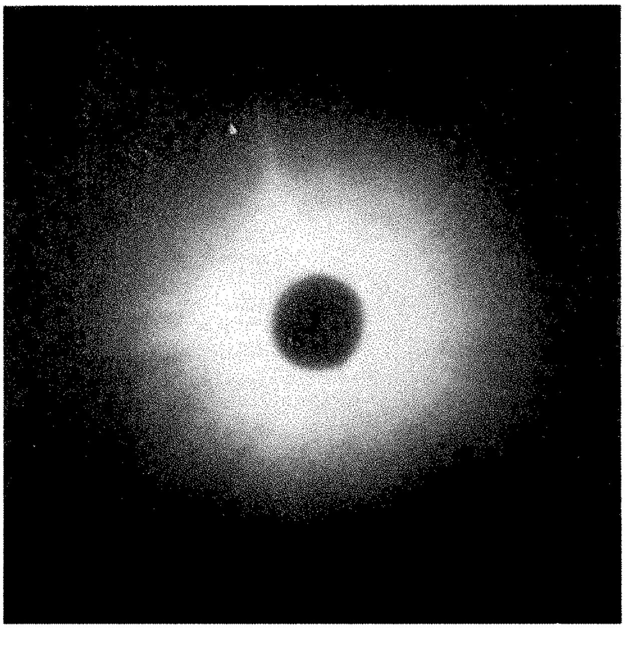

Fig. 1. Our calibrated photographs of the outer corona show streamers extending beyond 10 solar radii. This print, which shows more structure in the medium corona, is one of a series with stepped exposures.

out to at least 10 solar radii from the limb. They were long and straight, apparently unaffected by the curvature that solar rotation might induce. They also exhibited strong polarization.

Because the F-corona should be symmetrical around the limb, structureless and unpolarized, the character of these streamers and many other shorter rays indicates that they comprise the K-corona. Their polarization also supports this conclusion.

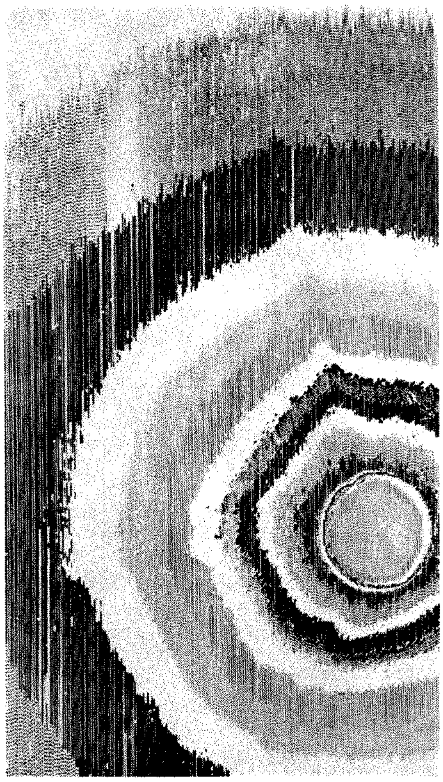

Fig. 2. Preliminary isophotes for the eclipse of March 7, 1970. Each change in the output of the isodensitracer—dashes to dots, dots to blank, and so on—corresponds to a change of 0·2 in photographic density.

Preliminary photometry of the records indicates that the streamers, however prominent they may seem to the eye, represent only slight increases of photographic density. Although the isophotes are not at all circular on Fig. 2 out to at least four radii, the streamers show up only as slight projections. This result indicates that they decrease in intensity away from the Sun at approximately the same rate as the overall coronal radiation. According to theory, the F-corona should decrease more slowly than the K-corona, so we conclude that inner zodiacal light does not contribute appreciably to the corona out to distances of at least 10 solar radii. As for ground-based observations of the Fraunhofer spectrum of the outer corona reported for previous eclipses, we suggest that it may well derive from cause (4): superposed skylight. To test this point, the slit of our spectrograph was long enough to cross the entire solar disk, so that any sky spectrum from the Moon's silhouette could be used for calibration.

The expedition was sponsored by the Harvard College Observatory, Smithsonian Astrophysical Observatory, National Geographic Society, Air Force Cambridge Research Laboratories, National Science Foundation, Videonics, Inc., and friends of the Harvard College Observatory. Other members of the expedition included F. A. Budreski, A. S. Clarke, D. L. Fernald, J. R. Hamilton, L. A. Lief, M. Mattei, J. Pitts, D. H. Read, F. de Romaña and W. W. Salisbury.

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Variations in Line Profiles from Photosphere to Chromosphere

WE observed from Miahuatlan (Oaxaca), Mexico, near the centre line of totality. A high-resolution Littrow-type grating spectrograph, fed by a coelostat and an objective lens (25 cm, 340 cm), was provided at second contact with a slit 12 μm wide, slanting through the last crescent of the solar photosphere and the chromosphere. The slit was removed before third contact.

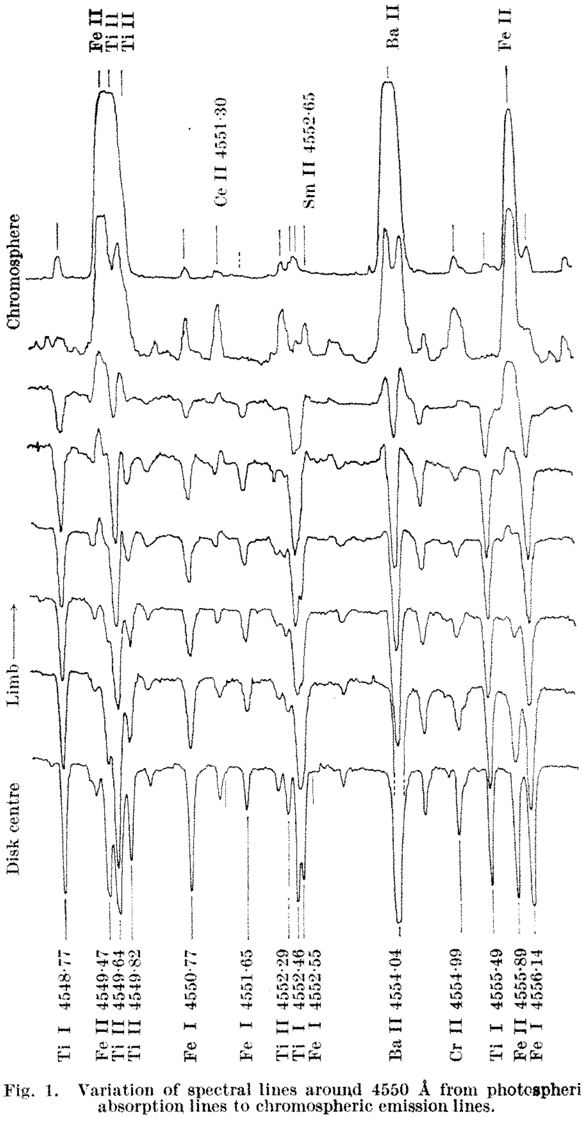

Fig. 1. Variation of spectral lines around 4550 Å from photospheric absorption lines to chromospheric emission lines.

We photographed the region from 4545 Å to 4579 Å simultaneously on two 70 mm films with respective intensities of 8 and 90 per cent, using a beam splitter, at a dispersion of 1·7 mm Å^{-1}. A shutter was guided photoelectrically in order to obtain the proper exposure times. Before and after the eclipse we took calibration spectra of the central part of the solar disk. In addition to this in the same spectrograph the region of the Mg b lines near 5200 Å was focused on a multiplex chopper and the coded spectrum was recorded on magnetic tape. This part of the experiment was developed by Th. de Graauw.

We made provisional microphotometric records in transmission of the photographic spectra at second contact. Because of differences in photographic densities and the adjustment of the photometer, the records show only qualitative differences in the appearance of spectral lines of different atomic and ionic origin in the transition from photosphere to chromosphere (see Fig. 1).

The extensive reduction of these spectra, using the laser-determined instrumental profile, the slit-jaw films and event-timing and time signal tapes, will take some time and the reduction of the multiplex tape has not yet started. Eventually we hope to obtain true line profiles of the outer limb and the transition region to the chromosphere.

We thank Drs C. de Jager and C. Zwaan for discussions and Messrs J. W. van den Broek and H. J. van Amerongen for help with the experiment. The expedition was supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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Association of Coronal Structures with Chromospheric Structure

SIX direct photographs of the total phase of the solar eclipse of March 7, 1970, were made at Sandbridge, Virginia (longitude 75·9161° W, latitude 36·6759° N, mid-totality 1836 UT), using an achromatic doublet of focal length 7·62 m and Eastman Kodak Tri-X orthochromatic sheet film. This note describes the coronal structures and their apparent relationship to chromospheric features observed in Hα and CaII K at the McMath-Hulbert Observatory (Table 1). We use the terminology suggested by Newkirk[1]; position angles are measured (accuracy ±3°) eastward from the pole of rotation; McMath plage numbers designate active centres; longitudes of disk features are given relative to the central meridian of the Sun.

Fig. 1 shows one of the photographs, and a schematic sketch locating the more significant features is presented in Fig. 2. Table 1 lists those coronal features most prominent on our photographs and their probable association with activity in the chromosphere. The quoted position angles of streamers are the positions of intersection of their axes with the limb.

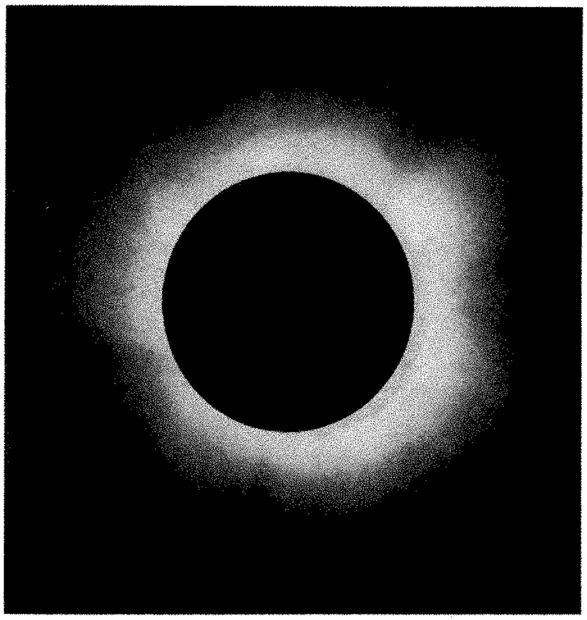

Fig. 1. This 20 s exposure beginning at 18 h 37 m 06 s UT was taken at f/30. Heliographic north is approximately at the top and east is to the right (see Fig. 2).

Especially noteworthy is the position angle of the cleft directly in the south and the decided difference in the appearance of coronal structure within about 30° (P.A.) of each pole. The western and northern parts of the corona, from P.A. 228° to P.A. 20° seem to be constituted predominantly of fine filamentary details (narrow rays) while the eastern and southern regions tend to be less filamentary and smoother in appearance. Streamers are without exception tilted poleward, towards the nearest pole, by varying amounts, contrary to the sense of tilt of streamers at minimum solar activity[2].

The maximum dimensions of the arches given in Table 1 are as estimated on our photographs. Such features have a subtle appearance in white light on low-contrast emulsions and are of somewhat uncertain extent. The arches at P.A. 30° and 190° are only partially visible on our photographs, and the quoted positions refer to the approximate centres of the arches as if they were complete.

An Hα filtroheliogram taken at 1631 UT is shown in Fig. 3 to aid in identification of structures on the visible

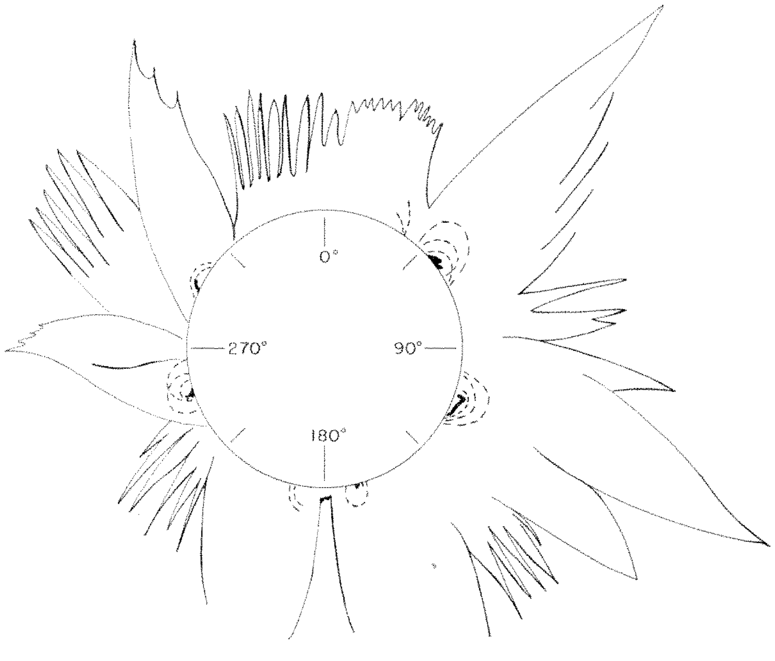

Fig. 2. The principal features shown on direct photographs are located in this sketch. Position angles are measured eastward from the north pole of rotation. Regions in which narrow rays were predominant are only schematic.

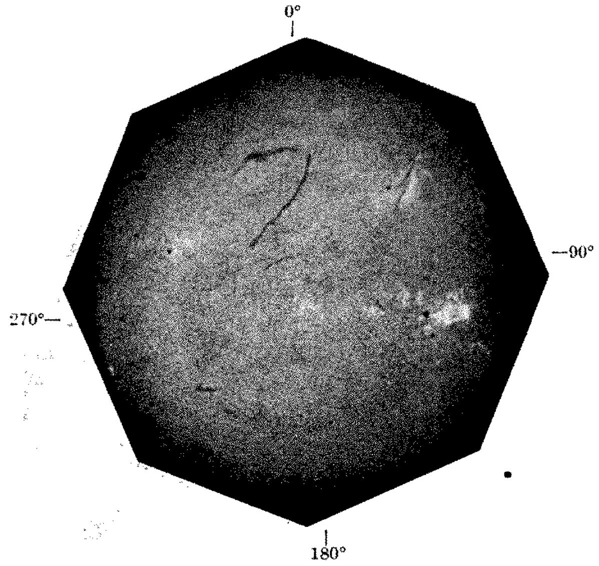

Fig. 3. This Hα filtroheliogram was taken at the McMath–Hulbert Observatory at 16 h 31 m UT. An imp. 1 flare was in progress in plage 10618 (S 14° E 42°). Cardinal directions on the Sun are given relative to the poles of rotation. The north pole was tilted 7·25° away from the Earth.

disk. Trending generally north-eastward from plage 10617 on the visible hemisphere is an extensive, well-developed filament which passes across the limb as a prominence near P.A. 54°. The major helmet streamer and arches near P.A. 50°–52° are associated with the plage or, more likely, with the prominence and filament. Observations on March 8, 9 and 13 show that behind the limb the filament jutted northward to at least P.A. 35°. It is possible that the amorphous portion of the northern "plume zone" near P.A. 20°–36° is associated with the north-eastern extension of the prominence. Hα filtroheliograms made on March 8 and 9, however, show the presence of a small high-latitude filament in the north-east which would have been on or close to the limb near P.A. 30° on the eclipse day. It may have been associated with the (partial ?) arches at that position angle.

Significant flaring activity occurred on March 7, 1970. Six subflares were reported in the plage on the south-west limb, No. 10621 (S 19°, W 80°) between 0 h and 18 h 30 m. Two 1N flares were reported after our time of totality. This plage formed on the previous day and a small spot was seen in it on March 7. A prominence at P.A. 250° is undoubtedly associated with this region. Above the prominence but not concentric with it is a system of arches, enclosed within a compact streamer that does not have a helmet-like appearance. Very extended, curving narrow rays meet the limb at position angles within the compact dome-like streamer. The plage region was not seen when the heliographic position which it occupied on March 7 next rounded the east limb, however. It is also possible that the compact coronal streamer is associated with plages 10602 (S 14°, W 80°) and 10604 (S 03°, W 70°).

On the day before the eclipse, March 6, a proton flare may have taken place behind the west limb. An eruptive bright surge was seen at P.A. 282°, beginning at 0926 UT, accompanied by a large 10 cm radio burst and followed by a loop prominence display and the detection of 20–70 MeV protons in space near the Earth. On our photographs the region of the corona near the position angle of the bright surge is confused, apparently consisting of a weak streamer on which narrow rays are superposed.

Table 1. POSITIONS AND DESCRIPTIONS OF CORONAL STRUCTURES

P.A.	Feature	Remarks
		Polar regions
330°–36°	Northern plumes	Rays very long and narrow. Somewhat amorphous in P.A. 20°–36°.
30°	Arches (partial ?)	Height ~3·4′ arc. No prominence within.
147°–228°	Southern streamers	Each has arches at base.
168°	Arches	Height ~2·3′ arc. Small prominence at base ?
180°	Cleft	6° broad at limb.
190°	Arches (partial ?)	Height ~2·4′ arc. No prom. within. Associated south-western streamer may be related to filament extending SE from plage 10584 (S 15° W 173°) nearly to limb in P.A. ~225°.
		Lower latitude regions
50°	Major helmet streamer	Associated with plage 10617 (N 19° E 30°) or with filament extending NE from it. Arches and prom. within. Axis is tilted 11° northward from radial direction.
52°	Arches	Height ~5·5′ arc. Width at base 22° P.A. Not concentric, not centred on prom. at P.A. 54°.
54°	Prominence	Extremely long prom. trending across NE limb.
83°	Streamer ?	A weak streamer may be present with a small prom. at its base at P.A. 79°.
94°	Streamer	No arches or prom. at base. Tilted 22° southward from radial. May be associated with plage 10618 (S 14° E 42°).
114°	Arches	Height ~3·9′ arc. Width at base 22° P.A. Higher arches not concentric, lowest arch centred over prom. at P.A. 116°.
120°	Streamer	Not well-defined. No arches or prom. within. Tilted 11° southward of radial. May be associated with plage 10629 (S 16° E 116°) and prom.
135°	Narrow rays	Prom. at base in P.A. 140°, no arches. Tilted 14° southward of radial.
230°	Narrow rays	The boundaries of this region in which there is an absence of bright streamers curve strongly away to north and south.
255°	Arches	Height ~2·9′ arc. Width at base 22° P.A. Higher arches not concentric; lowest arch centred over prom. at P.A. 250° in plage 10621 (S 19° W 80°). This is a new plage.
263°	Streamer	A compact streamer. Extended curving rays reach limb within base cf this streamer.
281°	Weak streamer with superposed narrow rays ?	Not well-defined. No arches or prom. at base. The structures appear generally to be tilted 32° northward of radial. See text.
300°	Arches	Height ~1·6′ arc. Width at base 12° P.A. These are concentric on P.A. 300°. A small prom. at P.A. 297° completes the southern arm of the inner arch. At location of plage 10598 (N 29° W 97°).
304°	Streamer	Tilted 39° northward of radial. May be associated with plage 10595 (N 16° W 110°) and/or with plage 10592 (N 18° W 137°) and/or with plage 10598 (N 29° W 97°).

The two most prominent features on our photographs are the major helmet streamer at P.A. 50°, already discussed, and the bright structure near P.A. 290°–300°, just north of the bright surge of the previous day. The latter is apparently part of the southern edge of a streamer seen superposed with other structures and the axis of which meets the lunar limb in P.A. 304°. There are three possible plage candidates for the chromospheric origin of this strongly tilted (39°) streamer (see Table 1).

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[1] Newkirk, jun., G., *Ann. Rev. Astron. Astrophys.*, **5**, 213 (1967).

[2] Van de Hulst, H. C., *The Sun* (edit. by Kuiper, G. P.), 297 (Univ. Chicago Press, 1953).

Photospheric Magnetic Fields on March 7, 1970

DAILY magnetograms were obtained at Kitt Peak beginning two weeks before and concluding two weeks after the eclipse. Initiated by Dr Gordon Newkirk[1], the aim of this programme was to produce a synoptic map of solar magnetism from which the potential field configuration might be inferred for comparison with observed coronal features (see Fig. 1). These results will be reported elsewhere by

Newkirk. A side product was the production of full disk magnetograms having a resolution of 2–3 arc s and a good response to a wide range of field intensity including that of sunspots. A new instrument, the 40-channel magnetograph, was available for these observations. In this note we present a magnetogram for the eclipse day (for comparison with other disk phenomena) and also magnetograms taken approximately one week before and after the eclipse day. The latter may be of some value for the interpretation of limb observations although the evolutionary change with time makes any comparison inexact.

The 40-channel system is essentially a forty-fold expansion of the Babcock magnetograph[2]. The 80 cm image of the McMath telescope is scanned in a TV raster pattern across the entrance slit of the spectrograph. In front of this slit is the usual electro-optic analyser for circularly polarized light. At the spectrograph focus a fibre optic probe dissects the spectral image of the wings of Fe 5233 Å, perpendicular to dispersion, into forty adjacent elements of 1 mm. Light is conducted from the probe to an array of eighty photomultiplier tubes which are followed by associated amplifiers and phase-locked detectors. Magnetic and brightness signals are recorded on magnetic tape. A full disk magnetogram, consisting of $\sim 6 \times 10^5$ elements, requires forty-five minutes to run.

Figs. 2, 3 and 4 show the magnetic data as intensity maps drawn by the computer. Positive fields are shaded light, neutral fields are grey and negative fields are dark. The response of the magnetograph to **B** cosγ is linear to ±2 per cent from 0 to 4,000 G. For these maps the relation between exposure *E* and **B** cosγ is given by

$$|\mathbf{B}\cos\gamma| < 10\ \mathrm{G},\ E = 56\ \text{(arbitrary units)}$$
$$-10\ \mathrm{G} > \mathbf{B}\cos\gamma \geq -2{,}000\ \mathrm{G},\ E = -108\ (\mathbf{B}\cos\gamma)^{-0.286}$$
$$+10\ \mathrm{G} < \mathbf{B}\cos\gamma \leq +2{,}000\ \mathrm{G},\ E = 29\ (\mathbf{B}\cos\gamma)^{+0.286}$$

Thus the weak fields are emphasized and a peak field

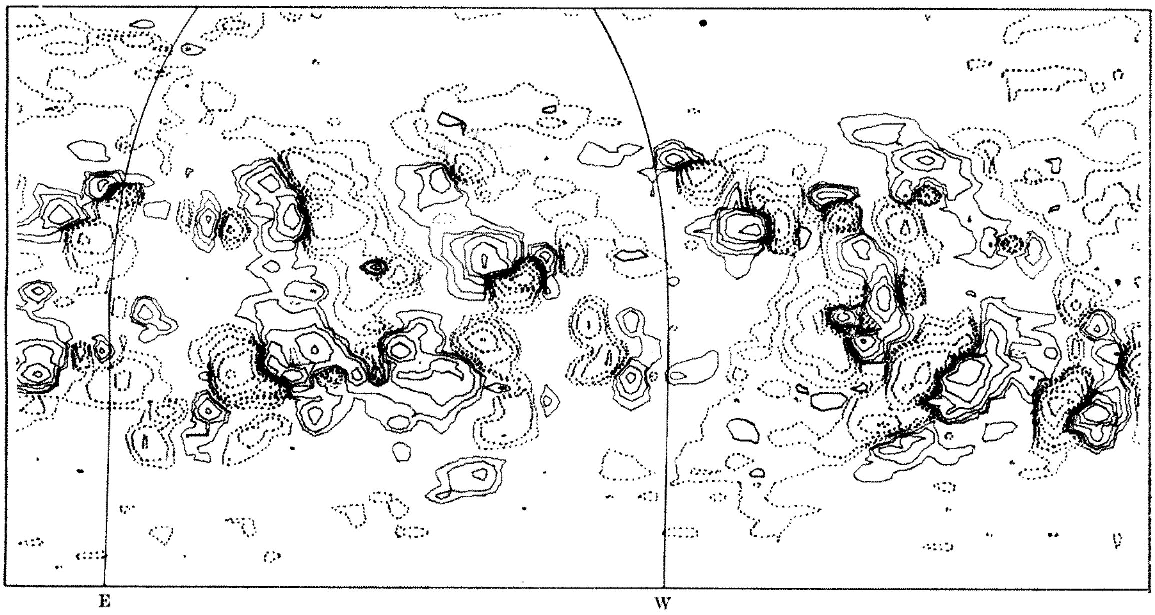

Fig. 1. Synoptic map of the average line of sight component of the magnetic field centred on the epoch of March 7, 1970. Solid and dashed contours enclose positive and negative magnetic flux respectively. North is at the top and east is to the left. The position of the limb at the time of the eclipse is indicated. The contours are drawn for values of ±4, 8, 16, 32, 64 and 128 G averaged over areas of 0·0417 in sine latitude and 6·6° in longitude.

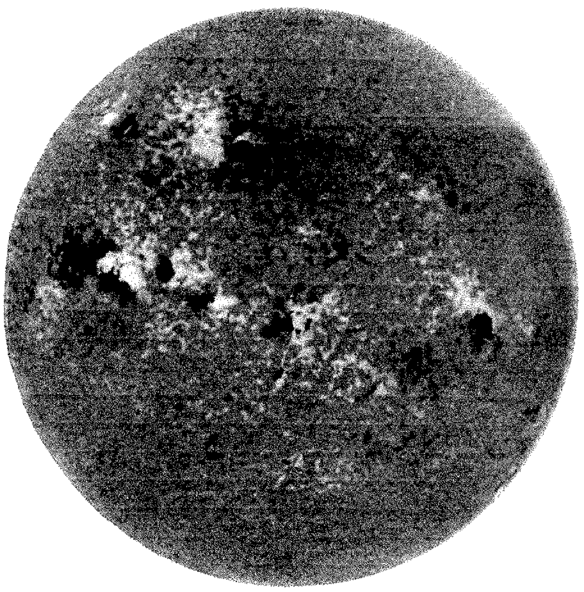

Fig. 2. Magnetogram for March 7, 1970 (2058 UT). Geocentric north is at top, west at the right. Ranging from bright to dark the steps are 2,000, 620, 200, 55, 14, −29, −110, −290, −750, and −2,000 G.

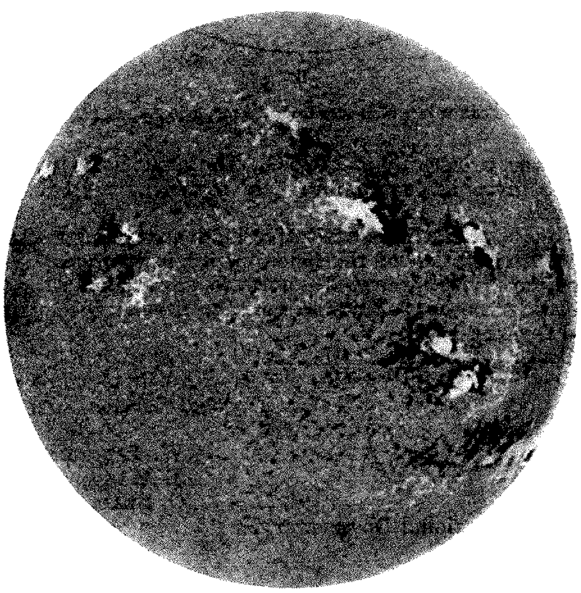

Fig. 3. Magnetogram for February 27, 1970 (1514 UT).

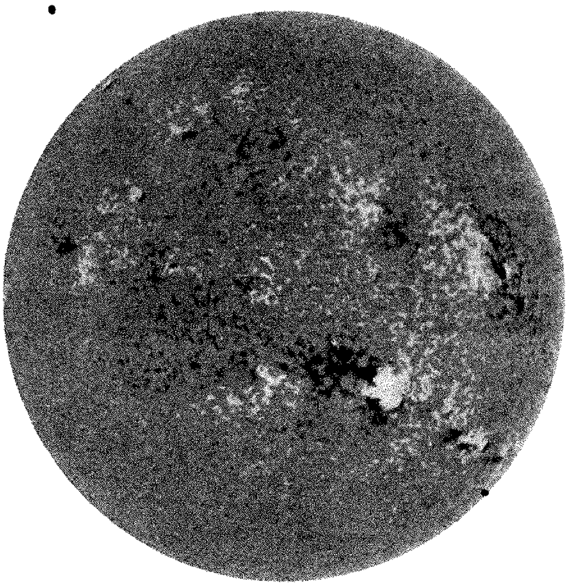

Fig. 4. Magnetogram for March 12, 1970 (1647 UT).

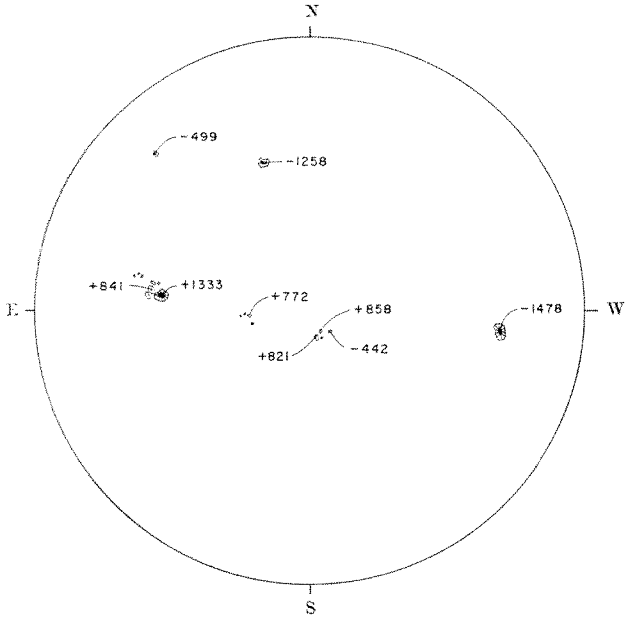

Fig. 5. Peak values of B cosγ from the magnetogram of March 7.

limit is set arbitrarily at 2,000 G. The r.m.s. noise is 10 G. Fig. 5 gives the maximum field strengths for sunspot umbrae.

We recognize the quantitative inadequacy imposed by the representation of the fields by intensity maps. We therefore invite interested parties to correspond with us regarding the availability of contour (isogauss) maps of local regions.

Kitt Peak National Observatory is operated by the Association of Universities for Research in Astronomy, Inc., under contract with the National Science Foundation.

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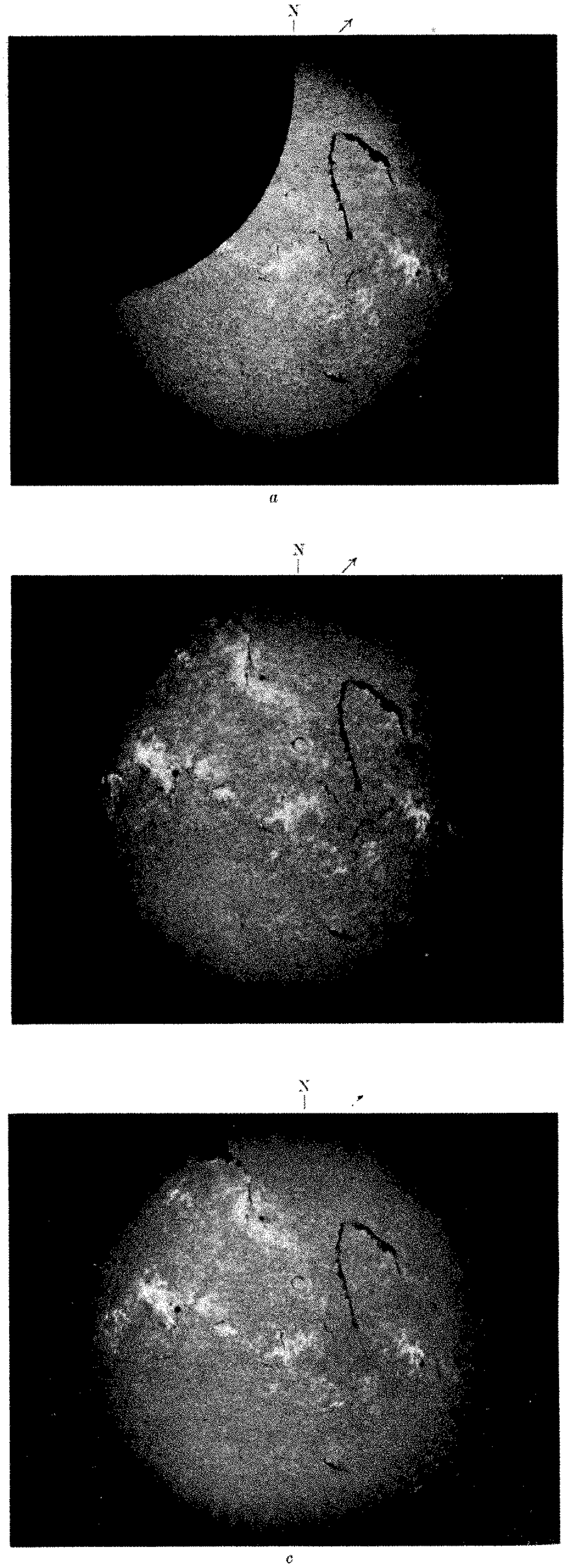
[1] Altschuler, M. D., and Newkirk, G. A., *Solar Phys.*, **9**, 131 (1969).

[2] Livingston, W. C., *Astrophys. J.*, **153**, 929 (1968).

Hα Filtergrams of the Sun on March 7, 1970

We obtained a series of Hα filtergrams of the Sun between 1835 and 1950 UT, during and subsequent to the eclipse.



a

c

Fig. 1. *a*, and *c*, Hα filtergrams during the eclipse of March 7, 1970. Arrows show the direction of the axis of rotation of the Sun.

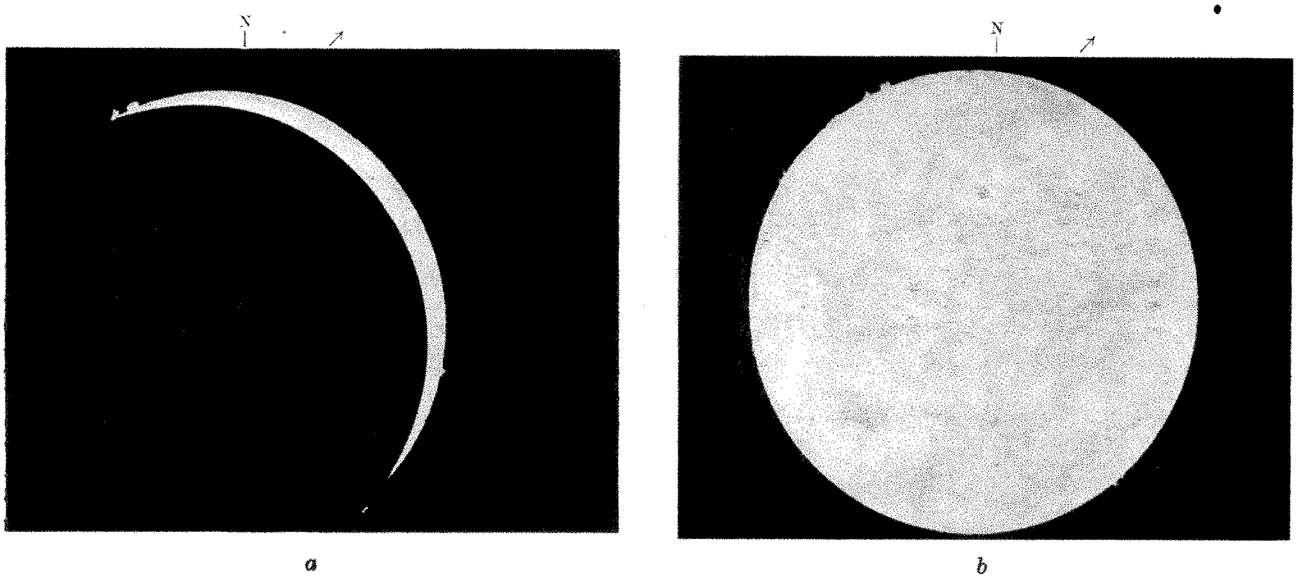


Fig. 2. *a* and *b*, Over-exposed Hα filtergrams during the eclipse of March 7, 1970. Notice the prominence beyond the limb of the Moon in the lower right side of Fig. 2*a*.

Although our observatory near Greenbelt, Maryland, was not in the path of totality, the photographs will be useful to others in interpreting observations of the eclipsed Sun.

The filtergrams were taken on Eastman SO-392 solar recording film with a Zeiss telescope of 96-inch focal length and a 0·5 Å Halle interference filter[1].

Figs. 1*a*, 1*b* and 2*a* show filtergrams during several phases of the eclipse. Figs. 1*c* and 2*b* are filtergrams made just after the fourth contact of the eclipse. Filtergrams 2*a* and *b* are over-exposed in order to show prominences. The bright plage area near the SW limb is particularly interesting, and this region at W 82° S 22° (region 72) was the site of a 1 N flare at 2120 UT (ref. 2). There were also several subflares and considerable surge activity associated with this region before, during and after the eclipse. A prominence that was possibly associated with this bright plage is shown clearly in Fig. 2. In Fig. 2*a* the Moon is still occulting the limb of the Sun, but the prominence is already visible.

A 1 B flare and a 1 N flare occurred at 1127 and 1607 UT respectively in another plage area at E 36° S 14° (region 87).

A striking filament, apparently greater than 500,000 km in length projected on the disk, is seen in Fig. 1.

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[1] Brandt, J. C., *Solar Phys.*, **6**, 490 (1969).

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Observations at the Sagamore Hill Solar Radio Observatory

THE solar eclipse of March 7, 1970, was observed at the Sagamore Hill Solar Radio Observatory, Hamilton, Massachusetts, with all the equipment normally used in the solar radio patrol[1]. The Sun was monitored with radiometric receiving equipment and associated antennas at frequencies of 114, 245, 606, 1,415, 2,695, 4,995, 8,800, 15,400 and 35,000 MHz. This is the first time that an eclipse has been observed at one site with such an array of equipment managed by one organization, with benefits in data consistency between frequencies, reliability and accuracy of calibration. At the site the eclipse was 96·2 per cent total.

Solar eclipses are still the best and least expensive means of studying certain characteristics of solar emission. This is especially true in the radio domain where a number of parameters can be studied which relate to the distribution of radio brightness across and beyond the disk. Several dozen eclipses have been observed by radio astronomers in the previous twenty-five years[2], and the emphasis at present is on solar predictions and beyond that, explanation and prediction of ionospheric activity.

This letter discusses the radio spectrum and history of one active region, McMath 607. We also consider the residual emission at eclipse maximum and discuss these spectral data in terms of changes in the solar spectrum during the sunspot cycle.

The eclipsing action of the Moon provides a resolution in one dimension which cannot be approached by even the newest and most sensitive interferometers. The extent, intensity and spectra of regions can be determined from eclipse occultation curves (Fig. 1) or more precisely from the derivative of the occultation curves.

Region No. 607 at about N 7°, W 26° early on March 7 was only one of four or five significant regions on the disk on that day. Regions 617 and 618 on the eastern part of the disk were considerably more complex and will be analysed in detail in a later paper. Region 607, however, was somewhat isolated and lends itself to unambiguous resolution during both the covering and uncovering. From February 27, when the region first appeared near the east limb until it disappeared on March 11 beyond the west limb, its potential for producing important flare-bursts diminished greatly. The day-to-day development of the region is shown in Table 1. Although the plage continued to increase in area during passage across the disk, the sunspot area and magnetic configurations were evolving into smaller and less important classes. Many subflares and a few major flares with associated radio bursts were observed in the early days of the passage. The optical flare-radio burst correlation also continued to weaken with passage across the disk.

It is not known what its flux spectrum was on those days, although we suspect that it favoured shorter wave-

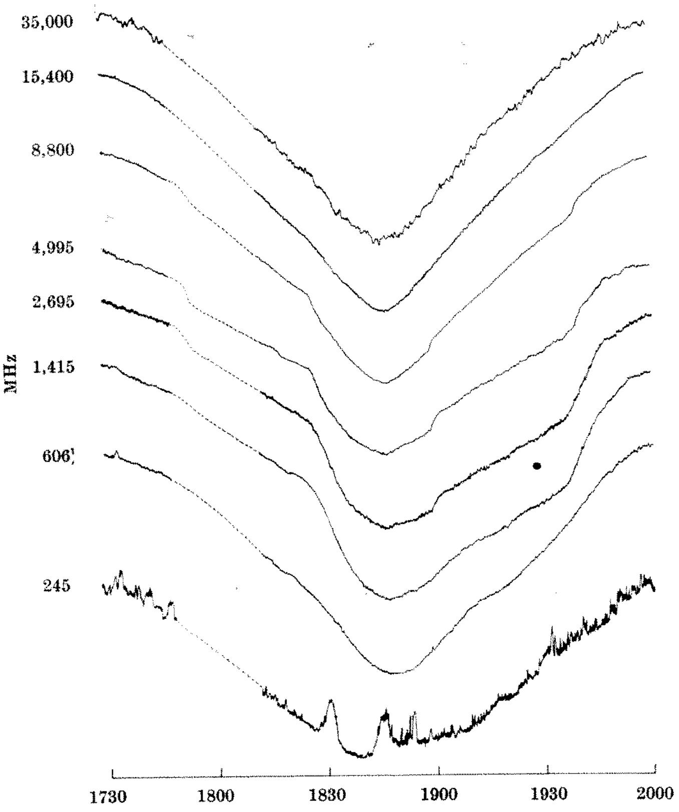

Fig. 1. Tracing of eclipse curves showing the covering and uncovering of strong sources of the slowly varying component of radio emission.

lengths. By March 7, the 9·1 cm brightness temperature had diminished greatly from its condition between March 1–5, 1970. The geomagnetic field, however, which became disturbed at 05/0805Z was probably related to the central meridian passage of this region.

The percentage changes in flux per minute of time caused by occultation of the radio source overlaying McMath Plage No. 607 are shown in Fig. 2 for the sources of wavelength 21·2, 11·1, 5·0, 4·29, and 3·4 cm for both the covering (ingress) and uncovering (egress) phases of the eclipse. Slope values are negative for the ingress and positive for the egress. The dashed lines indicate the slope values that would be obtained by eclipsing the background level of the Sun. To compress the figure, the ordinate values are arranged so that the zero level for the 11·1 cm curve is at the $-2·0$ level of the 21·2 cm curve, and so on for the other curves.

It can be seen that the changes in flux are more sharply defined and less extended in time, or dimension, during the uncovering of the source than during its covering. Optically the underlying plage and sunspot regions were rather extended longitudinally. The east–west extent of the spots was about 11° with the major spot group at the far west of the other spots in the group and at the western edge of the plage of 17° longitudinal extent. Because of this asymmetry of the optical region and the different geometry of the lunar limb during covering and uncovering, the non-symmetrical effects noted on the slope curves could be expected. But one effect that was not expected was that the shorter wavelength sources would be occulted first. Usually for a source with a radial height distribution as a function of wavelength located on the western sector of the Sun (the main spot group was at N 6° and W 26°), the longer wavelengths and thus the highest and most westerly sources would be eclipsed first. The same would be true for the egress where the longest wavelengths would be uncovered first. The slope curves for this region, however, show exactly the opposite. This apparent non-radial distribution could be accounted for by a shifting of the centre of gravity of the longer wavelength sources toward the east. These sources would then be more aligned with the centre of gravity of the plage.

Table 2. DIMENSIONS OF THE RADIO SOURCE ASSOCIATED WITH MCMATH PLAGE 607

Wavelength (cm)	3·4	4·3	6·0	11·1	21·2
Average source size (arc min)	0·8	0·8	1·1	1·6	3·1 ?

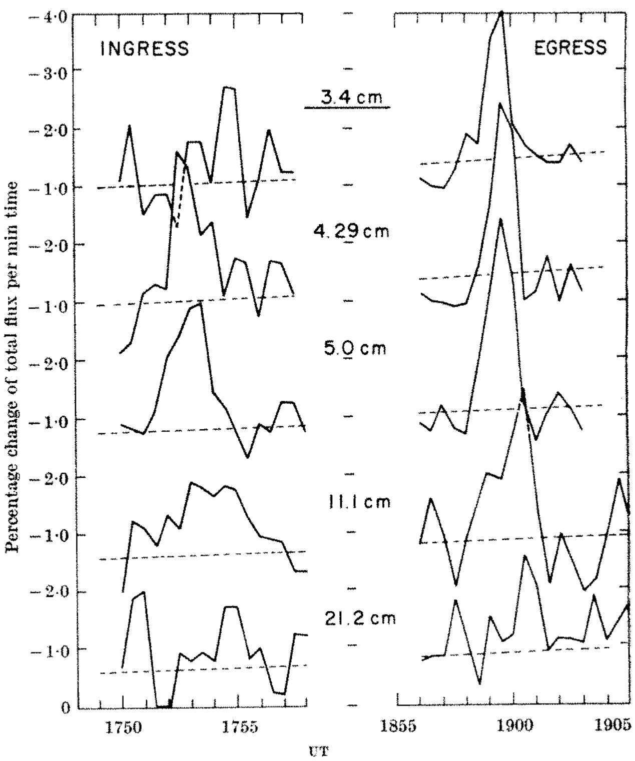

Fig. 2. Covering and uncovering of the radio source above plage 607 determined from occultation curves.

Table 1. ACTIVITY HISTORY OF MCMATH PLAGE 607

Date	Time of plage measurement	Plage position	Plage area (10^{-6} solar disk)	Zurich sunspot class	Sunspot area (10^{-6} solar disk)	Sunspot location	Sunspot magnetic class	9·1 cm T_b ($\times 10^4$ K)	No. of flares	No. of subflares
Feb. 27, 1970	0152	N6, E80	600	—	—	—	—	—	2	7
Feb. 28, 1970	0325	N8, E51	1,600	D	260	—	—	18	1	3
Mar. 1, 1970	0348	N6, E55	3,500	E	700	N7, E50	—	30	4	19
Mar. 2, 1970	0006	N6, E42	3,500	E	790	N7, E40	—	46	3	26
Mar. 2, 1970	2350	N6, E29	3,700	E	680	N6, E26	β, δ	25	2	14
Mar. 3, 1970	2355	N6, E16	4,400	E	610	N6, E11	β, δ	32	0	13
Mar. 5, 1970	0010	N6, E30	4,600	E	550	N6, W10	β, δ	31	0	9
Mar. 6, 1970	2353	N6, W10	4,600	E	390	N6, W12	β, δ	25	0	9
Mar. 7, 1970	0106	N6, W26	4,000	E	470	N6, W26	β	—	0	15
Mar. 8, 1970	0024	N6, W40	4,100	D	450	N7, W42	α	25	0	7
Mar. 9, 1970	0005	N7, W52	5,300	H	420	N6, W56	α	22	0	5
Mar. 10, 1970	0002	N8, W65	5,700	H	390	N6, W70	α	21	0	2
Mar. 10, 1970	2337	N8, W80	3,400	H	350	N6, W82	—	17	0	1

By measuring the time between half-power points of the region slopes and multiplying this by the average lunar velocity factor of 26·6 s of arc per minute of time, the source dimensions can be estimated. Table 2 shows the average dimensions from ingress and egress.

These sizes are small, probably because of the association of the source with the isolated alpha spot group. The dimensions at 21·2 cm are difficult to obtain from the slope records because of the low flux levels at this wavelength, but by extending the egress curve several minutes beyond 1905 UT, it was possible to see a suggestive drop of the slope to the quiet background. In any case, the dimension of 3·1 min has a possible uncertainty of ± 1 min.

Flux densities for the region at the various wavelengths were obtained by integrating the area under the curves. The resultant values in flux units (1 flux unit $= 1 \times 10^{-22}$ W m^{-2} Hz^{-1}) for the ingress curves, the egress curves, and for the averages of the two are given in Table 3.

Table 3. FLUX DENSITIES IN FLUX UNITS FOR NO. 607

Wavelength (cm)	3·4	4·3	6·0	11·1	21·2
Ingress	7·0	11·5	10·9	8·0	?
Egress	7·9	10·3	10·8	7·2	4·1 ?
Average	7·5	10·9	10·9	7·6	4·1 ?

The spectrum and low intensity of this source precluded its being resolved at all frequencies monitored. The flux values for the source are also shown in Fig. 3. The spectral curve resembles those of strong sources where the magnetic field introduces resonance absorption at harmonics of the gyromagnetic frequency[3-5].

Brightness temperatures for the region were determined from the measured flux values and the source sizes. Although some asymmetry in source distribution was observed, we assumed a circular distribution because of the apparent smallness of the sources and for simplicity of computation. Using the expression

$$T_b = \frac{3283\ F\ \lambda^2}{2k\ \Omega}$$

where F = source flux in W m^{-2} Hz^{-1}, λ = wavelength in metres, $k = 1{\cdot}38 \times 10^{-23}$ J (degree K)$^{-1}$ and Ω = source extent in square degrees, the brightness temperatures of Table 4 were obtained.

The brightness temperature at 21·2 cm may possibly be low because of errors in the flux, and especially the size at this wavelength. For example, if the source size had been 2·0′ arc, the brightness temperature would have been $2{\cdot}5 \times 10^6$ K.

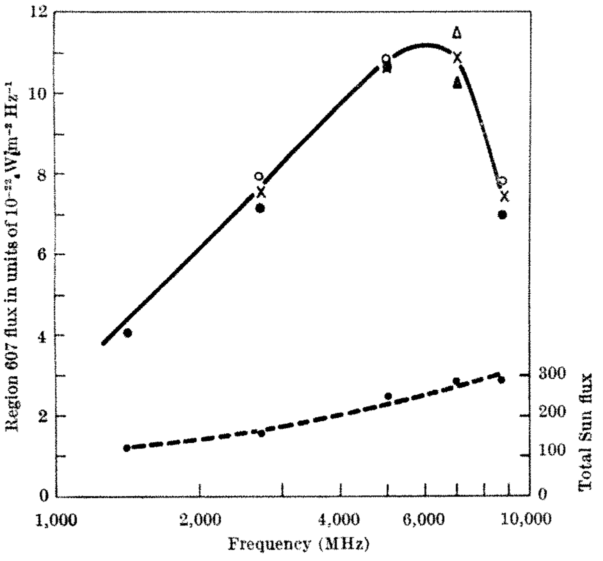

Fig. 3. Flux associated with McMath region 607. ○, Ingress values; ●, egress values; ×, averages; △, ▲, 4·29 cm data obtained by a group from Mackenzie University, São Paulo, Brazil.

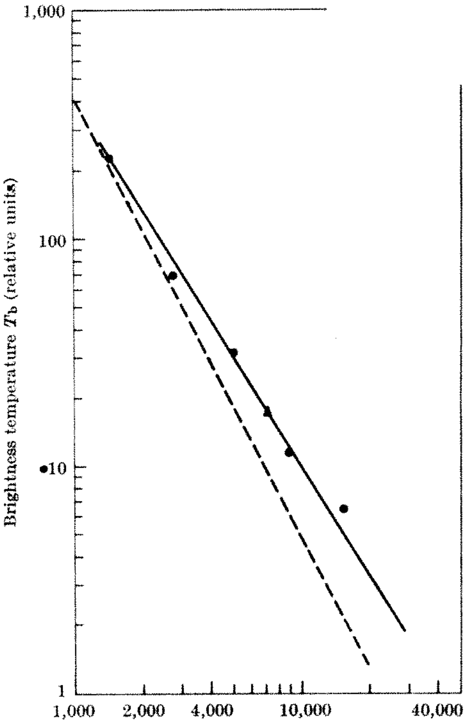

Fig. 4. Brightness temperature for the corona at centimetre wavelengths. The dashed line represents similar data for the eclipse of November 12, 1966.

The eclipse at Sagamore Hill was almost total. Hence it was possible to consider the residual flux at maximum phase as coming from beyond the solar disk. Using an approach similar to that applied by Kaufmann and Matsuura for the eclipse of November 12, 1966 (ref. 6), a plot of relative brightness temperature versus frequency for the residual was obtained. By this method the spectral index of the Sun at centimetre wavelengths can be approximated for different periods of the sunspot cycle. An attempt was made to normalize the values of the data for March 7, 1970, so that the two eclipses could be compared. The resulting plot is shown in Fig. 4, where the dashed curve is that given in Fig. 1 of ref. 6. The data from the 1970 eclipse have a flatter spectrum with a spectral index γ of $-1{\cdot}5$ ($T_b = af^\gamma$), compared with the index of $-1{\cdot}9$ for the 1966 eclipse. It is interesting that Tanaka and Kakinuma show that when the spectra of monthly mean values of the total solar flux are plotted as a function of period within the solar cycle, the spectra indeed flatten out during periods of high solar activity (paper at the Fifteenth General Assembly of URSI, Munich, 1966). For the mid-centimetre wavelengths, the Sun was considerably hotter on March 7, 1970, than on November 12, 1966.

Although the results are preliminary, it is apparent that the single source analysed, with its spectral peak near 6 cm, must be considered a representative source of the slowly varying component. It is possible that typical slowly varying sources are not important for burst production, but this requires further investigation. The non-radial distribution of the source under investigation seems real and confirms findings from at least one earlier eclipse[3].

We thank Drs O. Matsuura and P. Kaufmann for

Table 4. BRIGHTNESS TEMPERATURES OF THE SOURCE

Wavelength (cm)	Brightness temperature (K)
3·4	$7{\cdot}4 \times 10^5$
4·3	$1{\cdot}7 \times 10^6$
6·0	$1{\cdot}8 \times 10^6$
11·1	$2{\cdot}0 \times 10^6$
21·2	$1{\cdot}1 \times 10^6$

communicating preliminary data at 7,000 MHz recorded by the Mackenzie University group of São Paulo, Brazil.

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16 GHz Observations of the Eclipse

THIS letter presents the preliminary results of eclipse observations at a frequency of 16 GHz (1·9 cm) on March 7, 1970. The data define the diameter of the radio Sun at the 16 GHz observing frequency, and the initial analysis indicates non-uniform emission from both the limb and disk regions. The transportable radio telescope (Fig. 1) was located at Virginia Beach, Virginia (lat. 36° 50′ 19″ N, long. 76° 01′ 25″ W). It consists of a steel flatbed trailer which is raised on mechanical jacks when on site. A precision elevation over azimuth pedestal is employed. After rough levelling with the jacks, a rotating table and adjustable tilt base allow the pedestal to move in right ascension and declination. The offset paraboloid antenna, of aperture 40 inches, has a 1·5° beamwidth at 16 GHz. The first sidelobe levels are more than 30 dB below the main lobe gain. Two multimode feed horns are equally displaced in a horizontal plane by one half the horn diameter about the prime focus of the antenna. One beam is directed at the Sun and the other is $-3·5°$ from the Sun in right ascension. The receiver alternately samples the two signals at a rate of 1 kHz. The result of this two-beam technique is the subtraction of the atmospheric emission background, leaving only the signal due to the Sun.

The superheterodyne receiver has a mixer using a balanced Schottky barrier diode designed by W. M. Sharpless. The noise temperature is 1,200 K and the double sideband 3 dB bandwidth is 450 MHz. The signal was synchronously detected during the eclipse using a 12 s time constant and recorded on strip chart recorders. The output was linear within 2 per cent over a -20 dB range from the normal solar signal, with a signal resolution of approximately 1 part in 10^4. The observed fluctuations on the signal were due entirely to receiver gain variations and were approximately proportional to the signal level. The signal to noise ratio was approximately 75.

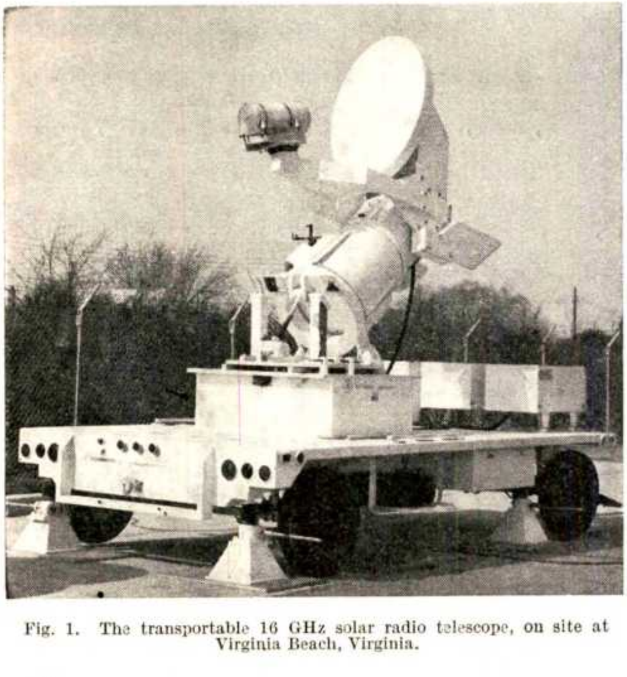

Fig. 1. The transportable 16 GHz solar radio telescope, on site at Virginia Beach, Virginia.

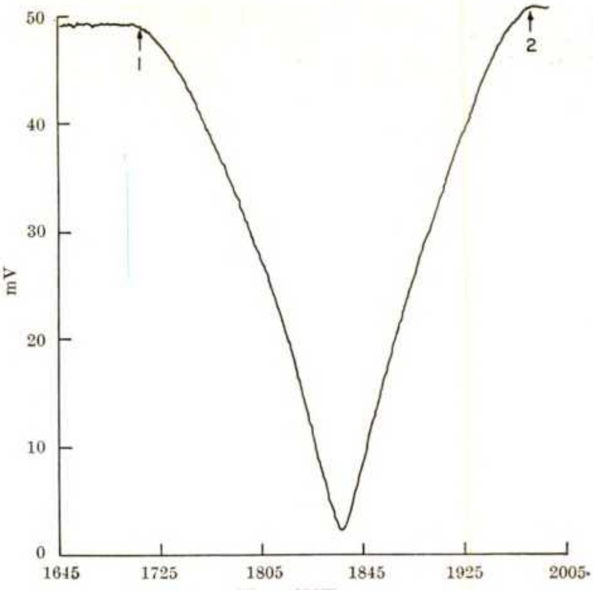

Fig. 2. The March 7, 1970, solar eclipse, as observed at 16 GHz. This figure is a computer representation of the original data and does not represent the inherent resolution of the experiment. Voltage values are linearly convertible to temperature, but the necessary parameter has not yet been derived. Points 1 and 2 indicate the beginning and end of the radio eclipse, as determined from the unsmoothed data.

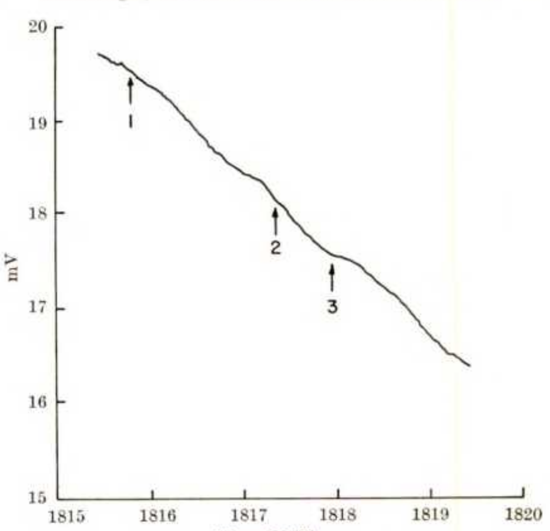

Fig. 3. The occultation of a large sunspot group by the Moon during the March 7, 1970, eclipse. Point 1 indicates the time at which occultation of a sunspot was optically observed to begin. Point 2 indicates the time at which occultation of a second sunspot began. The completion of the optical occultation is indicated by point 3. (See caption to Fig. 2 for comment on ordinate scale.)

The basic eclipse data at 16 GHz are shown in Fig. 2 and the points of first and fourth contact for the radio data are also indicated. The first and fourth radio contact occurred approximately 90 ± 10 and 85 ± 10 s before and after the respective optical contacts. This implies an average 16 GHz radio diameter for the Sun of $1·038 \pm 0·002$ times the optical diameter. The maximum of the radio eclipse occurred 18 ± 4 s after the optical maximum, implying an asymmetry of the radio Sun as a result of extended emission from regions on the NE limb. The larger values observed for the time derivative of the signal strength following the maximum radio eclipse as compared with pre-maximum values, along with the relatively early time of first radio contact, suggest that McMath plage region 10621, on the SW limb, was a small but bright source of emission at 16 GHz. The residual signal at maximum eclipse is 2·6 ± 0·2 per cent of the uneclipsed solar value, which implies a solar radio diameter of 1·039 times the optical diameter.

A detailed study of the data for the purpose of reconstructing the structural features of the radio disk has not yet been completed, but some preliminary results are available. Upon occultation of the large sunspot group at S15E40 (McMath plage region 10618), a decrease in the magnitude of the slope was observed, indicating the presence of a cool region on the disk. This is illustrated in Fig. 3. The occultation of the sunspot at W38N07 (McMath plage region 10607) has also been seen on the 16 GHz data.

Our preliminary results are summarized as follows: (1) The solar radio diameter at 16 GHz (1·9 cm) was 1·038 ± 0·002 times the solar optical diameter. This result is larger than that determined from a previous eclipse observation[1] by Shih-weh *et al.* at 18·8 GHz (1·6 cm) and approximately equal to previous determinations[1,2] at 10 GHz (3 cm), and may be indicative of the relatively high level of solar activity present during this eclipse. (2) The residual flux at maximum eclipse was 2·6 ± 0·2 per cent, in reasonable agreement with values derived from previous observations[3]. (3) Effects of sunspots and other non-uniformities were seen in the data. Detailed analysis of the structural features of the radio Sun is continuing.

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Observations with a 7 GHz Polarimeter

THE eclipse of March 7, 1970, was observed at 7 GHz with a polarization solar radio telescope at Sagamore Hill Radio Observatory (AFCRL), Hamilton, Massachusetts, where the magnitude of the eclipse was 0·96. We used basically the same equipment for the eclipse of November 12, 1966 (refs. 1–3). Two outputs were available, one proportional to the total solar flux ($S_R + S_L$), and the other to the difference in flux between the right and left-handed components of circular polarization ($S_R - S_L$). The degree of polarization is then the ratio of the outputs $(S_R - S_L)/(S_R + S_L)$. Solar activity was monitored before and after the eclipse date.

Fig. 1 shows the eclipse curve obtained after preliminary reduction of the data, where the time resolution was better than 20 s. Fig. 1 also shows the standard deviation of averaged values of the degree of polarization for each interval of 2 min.

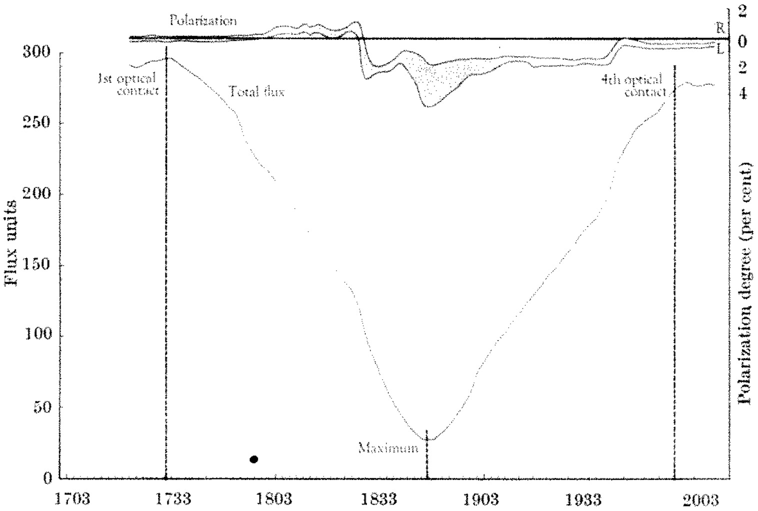

Fig. 1. The eclipse curve at 7 GHz, taken at intervals of 2 min. The upper curve refers to the total degree of circular polarization and represents the limits of the r.m.s. variations in intervals of 2 min.

Shortly before the eclipse maximum at ~18·45 UT, only the northern hemisphere of the Sun was visible, and the polarization was definitively left-handed. This result supports the possible emission of polarized radio waves from the northern solar hemispheres[3], but this is not certain because important coronal condensations which could influence the polarization effect were reported by optical observers.

Fig. 2 is the derivative of the eclipse curve[4], taken in intervals of 2 min of time, which indicates the presence of stronger active regions. At least two extended regions can be identified, one with intensity greater than 10 flux units and the other greater than 40 f.u. Most of the active regions were not strongly polarized, excepting region No. 2 (probably associated with McMath plage 10618 in the solar eastern hemisphere) which contributed 48 f.u. During the eclipse of this large active zone, a smaller region with strong right-handed polarization was covered and computations showed that its degree of polarization was about 10 per cent. It could have been the preceding part of a relatively complex sunspot group.

Burst activity at metric wavelengths was reported by AFCRL, Sagamore Hill, at about 1830 UT, close to the maximum of the eclipse. The activity did not appear at frequencies greater than 606 MHz, but it is possible that the S-component where such bursts originate did not remain stable during the eclipse, explaining some of the asymmetries in the eclipse derivative curve.

The residual flux at the maximum of the eclipse was nearly 8 per cent of the total solar flux and nearly 13 per cent of the quiet solar flux (deduced from the derivative curve and corrected for the contribution from the Moon), a figure larger than that obtained during the

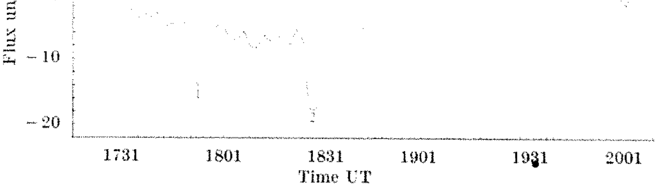

Fig. 2. The eclipse derivative curve, based on time intervals of 2 min.

1966 eclipse[2,5], because this time part of the solar disk was still visible.

The diameter of the radio sphere was not determined with great accuracy. Just before first contact the burst activity reported at 200 MHz could have influenced to some extent the activity at 7 GHz. From the more definite fourth contact and the eclipse derivative curve we expect the existence of detectable coronal emission at 7 GHz for heights lower than 1·2 solar radii.

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Radio Observations of the Distribution of Chromospheric Brightness Temperature

MODELS of the temperature and density distributions in the solar chromosphere may be tested by radio observations of the dependence of the brightness temperature on wavelength and position across the solar disk. The general increase in brightness temperature T_b at the centre of the disk with increasing wavelength of observation λ is well described[1] by the van de Hulst model[2] and also by the BCA model[3]. If such models are taken to be spherically symmetric, then the distribution of brightness temperature across the solar disk at a given wavelength is expected to increase toward the limb as sec θ, where sec $\theta = (1-\rho^2)^{-1/2}$, ρ being the position, in units of the solar radius, on the disk. The amplitude of the sec θ variation is determined by the dependence of the optical depth on height. Thus, although the $T_b - \lambda$ and $T_b - \rho$ distributions are interrelated, the $T_b - \rho$ distribution may be interpreted as an indication of the geometrical structure of the chromosphere in the sense that its functional form depends on the geometry. The earliest evidence of failure of the sec θ distribution, and hence failure of the assumption of spherical symmetry, came from the eclipse observations of Coates, Gibson, and Hagen[4] at 8·6 mm which revealed a flat distribution across the disk and a possible spike at the limb. More recently, high resolution observations (resolutions less than 3 min of arc) of the quiet Sun have revealed similarly flat distributions to $\rho \sim 0{\cdot}8$ over the range 3·5 mm to 2 cm (ref. 5). These observations and the recent observations in the ultraviolet have been interpreted by Simon and Zirin[6] in terms of a rough structure of the solar chromosphere (similar in spirit to the spicule interpretation of the 8·6 mm limb spike by Coates[7] and Athay[8]). Provided that the scale of roughness is small compared with the observational resolution, the rough surface would present the same mean brightness temperature at all but the most extreme aspect angles. Thus a narrow region of limb brightening close to the limb is to be expected. The high resolutions necessary to resolve structure near the limb can be attained during an eclipse, and a cooperative effort was organized among those observatories near the eclipse path which can observe at cm and mm wavelengths. The observatories taking part in the March eclipse were: the University of Texas at Austin (2·1 mm, Cogdell), NRAO at Kitt Peak (3·5 mm, Simon), Aerospace Corporation (3·4 mm, Shimabukuro), Haystack facility of MIT Lincoln Labs (1·35 cm, Zapata) and NRAO at Green Bank (2 cm, Buhl). Poor weather in western Texas prevented observations there, but good data were obtained at the other observatories. The data that we have analysed most completely so far are at 3·4 mm and 1·35 cm, and we shall restrict our discussion to those. (A similar effort was carried out during the annular eclipse of September 11, 1969, and data over the range 2·1 mm to 3 cm were obtained.)

The general technique was to position the axis of the main beam of the antenna on the first and last contact positions on the lunar limb and to track those positions as they crossed the limb of the Sun. In effect, the antenna beam was thus "sharpened" by the knife edge of the Moon. In between the times of first and last contact high resolution observations of other regions on the Sun were possible (within the obvious limitations of the local eclipse geometry) by tracking the appropriate position on the lunar limb.

The 3·4 mm data obtained at first contact are shown in Fig. 1. The data points were taken every 6 s with a time constant of 1 s (at times later than ~1633 UT we have displayed only every other data point). The data were corrected for atmospheric extinction and normalized to unity by averaging over the time interval from around 1650 to 1700 UT (during which the antenna was passing over $\rho \sim 0{\cdot}7$ on the solar disk). The short term scatter of the points is consistent with the 2–3″ tracking "jitter" of the antenna. The line through the data is an "eye" fit. At 1·35 cm the data were taken every second with a one second time constant. The 1·35 cm points in Fig. 2 for the last contact are 10 s averages, and the data were normalized to unity over the time interval from around 1932 to 1940 UT when the antenna was passing over $\rho \sim 0{\cdot}3$ to $\rho \sim 0{\cdot}5$. The scatter in these points is also consistent with the monitored antenna tracking errors and the line drawn through them is again an eye fit to the data.

Both these records yield information much nearer to the solar limb than has been available previously. For example, in Fig. 1, the antenna was at $\rho \sim 0{\cdot}97$ on the solar disk at 1630 UT and in Fig. 2 was at $\rho \sim 0{\cdot}95$ at 1955 UT. Inspection of Hα photographs of the Sun for March 7 reveals that in the regions covered by the scans represented in Figs. 1 and 2 there were no obvious active regions so that the scans are presumably representative of the quiet Sun. (There were, however, active regions close to the point of last contact at 3·4 mm and to the point of first contact at 1·35 cm which appear as brightness enhancements on those scans.)

The general roughness of the brightness temperature distribution is evident in both figures. The amplitude of

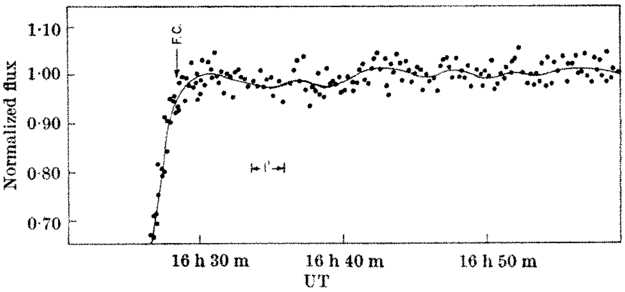

Fig. 1. First contact data obtained at 3·4 mm with the 15-foot telescope of the Aerospace Corporation. The time of first contact (F.C.) and one min of arc scale size on the Sun are indicated.

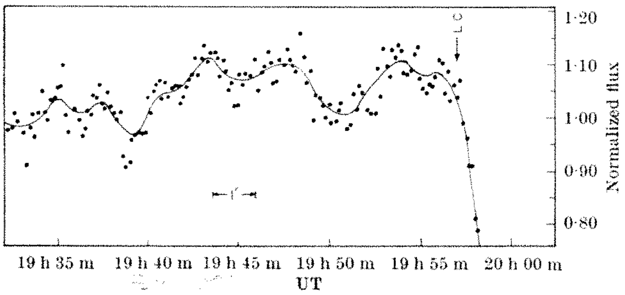

Fig. 2. Last contact data obtained at 1·35 cm with the 120-foot Haystack telescope of MIT–Lincoln Laboratory. The time of last contact (L.C.) and one min of arc size are indicated.

the fluctuations is much greater at 1·35 cm than at 3·4 mm, and in both cases the smallest half-width of the smoothed fluctuations of brightness temperature is about half a minute of arc. (The intrinsic, or "non-eclipse", half-power beam width of the Aerospace Corporation telescope used to obtain the 3·4 mm data was 2·8′, and 1·4′ for the Haystack antenna at 1·35 cm.) In our analysis of the 3·4 mm data from the eclipse of September 11, 1969, and also from the 3·5 mm data for this eclipse, we found that some of the brightness temperature dips correlate well with positions of filaments observed in Hα. This is not the case here, as there were no filaments near the scan tracks represented in Figs. 1 and 2. The general character of the 3·4 mm distribution in Fig. 1 is consistent with the extensive solar mappings at 3·5 mm carried out by Buhl and Tlamicha[9] with 1·2′ resolution. In those observations the distribution of brightness temperature for the quiet Sun appeared to have fluctuations on the scale of the instrumental resolution, and also there were indications that filaments were beginning to be resolved. It is interesting to note that the size scale of the fluctuations resolved in Figs. 1 and 2 is comparable to the typical size of the chromospheric network, and we may speculate that in fact the fluctuations are determined by the chromospheric network.

Allowing for the generally undulating nature of the 3·4 mm distribution in Fig. 1, it is seen that there is little, if any, direct observational evidence of limb brightening. By convolution of various models of limb brightening, we can place an upper limit of 1 per cent on the total power that might be contained in an annulus of limb brightening at the limb, and an upper limit of 0·03 $R_{\odot}$ on its width. These results are consistent with analysis of the 3·4 mm observations during the September eclipse.

There is clear evidence of limb brightening in the 1·35 cm observations from the generally enhanced brightness temperature at the time of last contact (compare also the rate of drop-off of the brightness temperature at the time of contact in the two figures). The enhancement prior to 1950 UT (when the antenna was at $\rho \sim 0{\cdot}8$) should not be considered to be limb brightening because Tlamicha's observations[5] showed that the distribution of the undisturbed Sun is statistically flat to at least $\rho \sim 0{\cdot}8$. Rather, we believe that the fluctuations observed at $\rho < 0{\cdot}8$ should be interpreted as contributing, when at the limb and foreshortened, to the enhancement observed at the limb.

The preliminary analysis of the data that we have carried out so far therefore indicates that fluctuations of brightness temperature of about 0·5 minute of arc in size on the undisturbed Sun have been resolved, and that their amplitude increases with the wavelength of observation. Their size suggests an origin in the chromospheric network.

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Effects on Migration of Marine Organisms in the Gulf of Mexico

THE research vessel Alaminos from Texas A & M University was in the Gulf of Mexico on March 7 to record changes in the near-surface layers of sea and air as a consequence of the eclipse. So that a maximum variation in incident solar radiation could be realized, the observing site was chosen at 24° N and 89·5° W where totality would occur approximately at local solar noon over waters deeper than 100 fathoms.

Among the aims of the cruise were measurements of the vertical migration of zooplankton in the near-surface layer, the depth of the deep-scattering layer, and indications of biological productivity.

A preliminary examination of acoustic reverberation records taken at 12 kHz revealed the existence of four or five deep scattering layers—layers of concentrated marine organisms—during the 3 days on the day before, during and after the eclipse. These were at depths of 20–250 fathoms. During the test period the layers showed the characteristic upward migration around sunset and downward migration at sunrise.

Several of these layers seem to have responded to the eclipse but only one displayed a definite and measurable reaction (Figs. 1*d* and 2*d*). This layer, at 80 fathoms on each day during the test period, migrated upward about 4 fathoms, but the migration lagged behind the eclipse by about 20 min. The layer also responded to several periods of relatively heavy cloud on the afternoon of the day of the eclipse and the following day. The evening upward migration seems to have started earlier (about 1500 h) on the day of the eclipse than on the day after (about 1730 h). This may have arisen from confusion in the layer, or a rougher sea on the following day, so that more light entered the sea in the later afternoon.

The near-surface zooplankton population was sampled by periodic 10 min tows at a standard wire angle with an array of 12 inch Clarke–Bumpus plankton samplers. Concurrent sampling at four depths from the surface to 75 m below was customary, although only surface and 25 m samples were obtained during totality.

An impression of the relative volume of plankton samples taken at these two depths during the 1·5 days of observation can be obtained from an examination of the settled material (Figs. 1*b*, *c* and 2*b*, *c*). We are reluctant to speculate on the significance of the data, but there does seem to have been a relative increase in the volume of zooplankton near the surface and a decrease at 25 m during the darkest moments of the eclipse. A similar increase is evident for the following evening and may represent upward migration of certain elements of the zooplankton community in response to decreased levels of light intensity. We note, however, that this does not seem to have been the case at these depths during the night before the eclipse.

Initial examinations of the samples reveal a predominance of copepods, euphausiaceans, chaetognaths and small decapods. But because observations of the vertical migration of marine zooplankters can be considered valid only in relation to individual species, meaningful conclusions must await further analysis.

During this cruise the standing crop of phytoplankton was estimated by determining the chlorophyll *a* content spectrophotometrically (using a D-U spectrophotometer) and fluorometrically (using a Turner fluorometer, model 111) during the period March 6–8, 1970. Continuous fluorometric monitoring of chlorophyll *a* in near-surface seawater (depth, 3 m) did not show discernible variations in the standing crop of phytoplankton at comparable times during the observations. Integrated chlorophyll *a* values of water samples collected in the water column from the surface down to 50 m at 1045 h on March 6, 7 and 8 were 17·72, 11·76 and 11·82 mg/m², respectively. Unfortunately, the data collected on phytoplankton are not sufficient to suggest a cause and effect relationship; other factors, grazing, for instance, could be implicated in explaining the decrease in the phytoplankton biomass during and following the solar eclipse day.

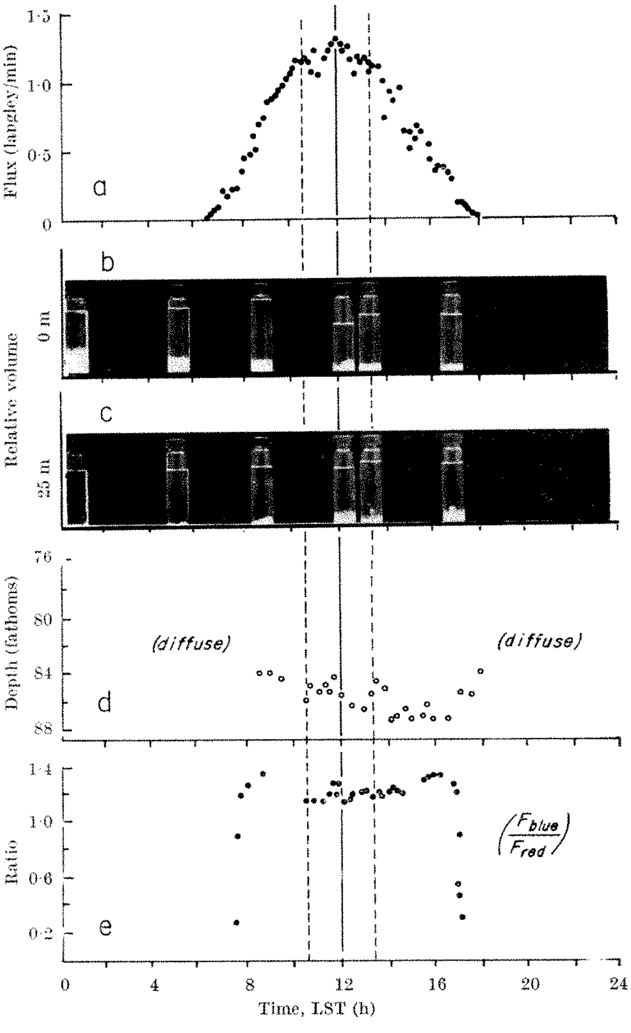

Fig. 1. Diurnal variations on March 8, 1970, of insolation, measured with a pyranometer which incorporated a silicon photovoltaic cell as sensor, mounted on the weatherdeck (*a*); relative volume of zooplankton near surface (*b*) or at 25 m (*c*); depth of a deep-scattering layer (*d*); and (*e*) ratio of blue to red solar radiation.

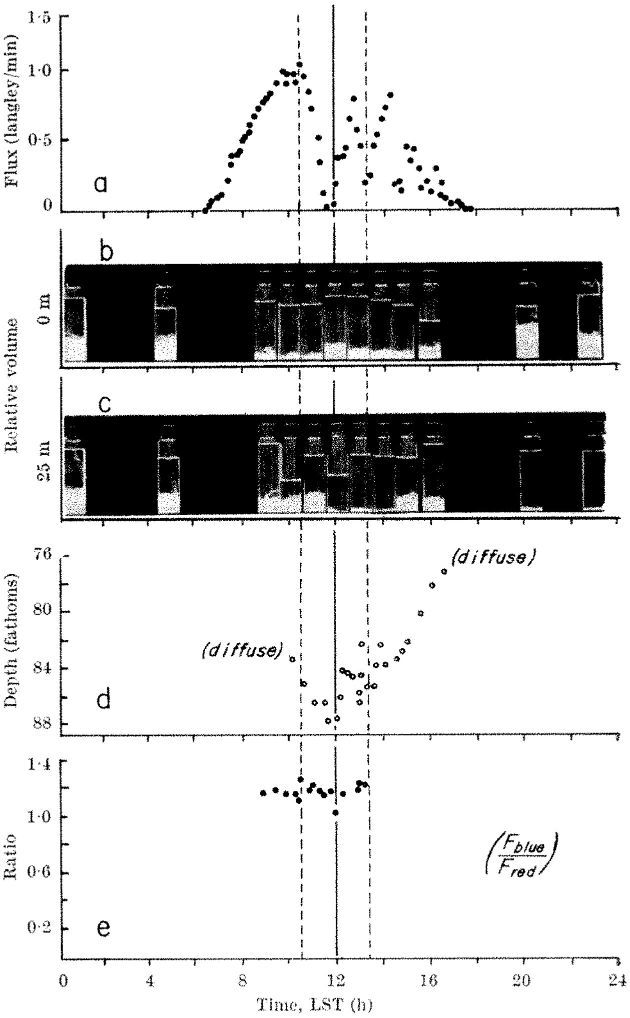

Fig. 2. Same as Fig. 1, but for the day of the eclipse, March 7. The sky was cloudy from 1530 to 1640 LST. When the solar disk was half covered, near 1127 LST, insolation was reduced to 40 per cent of the unobscured value for the same time on the following day. Presumably thin strato-cumulus clouds accounted for the additional 10 per cent reduction.

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Hormones in the Translational Control of Early Germination in Wheat Embryos

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Gibberellic and abscisic acid seem to exert their effects on germination by acting in some way at the level of translation. The two hormones may act at different sites.

THERE is at present much interest in two ways in which hormones may regulate the rate and pattern of growth and differentiation—the transcription of informational RNA from the DNA template[1] and the translation of informational RNA in processes of protein synthesis[2]. Both mechanisms may be operative in plant systems[3,4], but the continued transcription of RNA from DNA by most growing tissues makes precise interpretation of the data difficult. In germinating wheat embryos, however, the genome is essentially inactive for the first 12 h and no mRNA is transcribed[5]. Nevertheless, protein synthesis is initiated within 30 min of the embryos imbibing water, indicating that a functional informational and translational system is present within the tissue[6,7].

We have used wheat embryos to study the effects of two plant hormones, gibberellin A3 (GA) and abscisic acid (ABA), on the processes of protein synthesis. Both hormones regulate the growth of the embryos, GA as an accelerator of germination, and ABA as an inhibitor.

The effects of a range of concentrations of GA and ABA on the germination of wheat embryos were determined using the procedures described in Fig. 1. When 10^{-4} M GA was present in the germination medium, development of the embryos was advanced; at 12 h root emergence could already be detected and by 24 h fresh weight was 20 per cent higher than that of controls. Although barley embryos are considered to be a source of GA[8] which includes the *de novo* synthesis of certain enzymes in the aleurone of the endosperm[9–11], it is evident that added gibberellin also enhances the growth of the embryo[12]. When 10^{-4} M ABA was included both in the agar medium and in the germination medium, germination was inhibited, and the fresh weight was 28 per cent less than the controls.

DNA Synthesis

Both GA[13] and ABA[14] are reported to affect DNA synthesis, but it is not clear whether the hormones control the actual initiation of DNA replication. This was checked in wheat embryos by following the effects of the hormones on the incorporation of radioactive precursors into DNA. After 7 and 23 h germination in the presence of germination medium, or germination medium with GA, embryos were pulsed with ^{14}C-thymidine for 1 h before analysis. No radioactivity above background was found in the trichloroacetic acid (TCA) insoluble fractions labelled during 7–8 h of germination, nor in the isolated and purified DNA[7] (Fig. 1*a* and *b*). Moreover, during the period after which DNA replication is initiated (24 h), no stimulating effects of GA on the rate of DNA synthesis were observed. Clearly, the initiation of replication is not under direct control by GA. The results agree with those of Haber[15], who concludes that DNA synthesis is not a requirement for growth regulation by GA. By contrast, ABA depresses the rate of DNA synthesis once it has been initiated.

RNA Synthesis

If plant hormones function at the gene level[16] there must be induction or repression of specific gene products coding for the induced enzymes. Most evidence, however, indicates an overall stimulation of RNA synthesis in plants treated with hormones[17–20]. Because the synthesis of RNA in germinating wheat embryos is repressed during the first 12 h of germination[5], it should be possible in this system to detect an earlier RNA synthesis induced by hormones. Embryos germinated in GA and ABA were therefore pulsed with ^{3}H-uridine for 1 h between 11 and 12 h of germination. The values for ^{3}H-uridine incorporated, as percentage of total uptake, show that RNA synthesis is switched on only after 12 h of germination, irrespective of the presence of GA or ABA (Fig. 2). The level of these analyses does not preclude the possibility that a small and specific functional RNA is included in the early hours of germination. But clearly, by 24 h, the rate of total RNA synthesis is enhanced by 22 per cent in embryos treated with GA and reduced by 49 per cent by ABA.

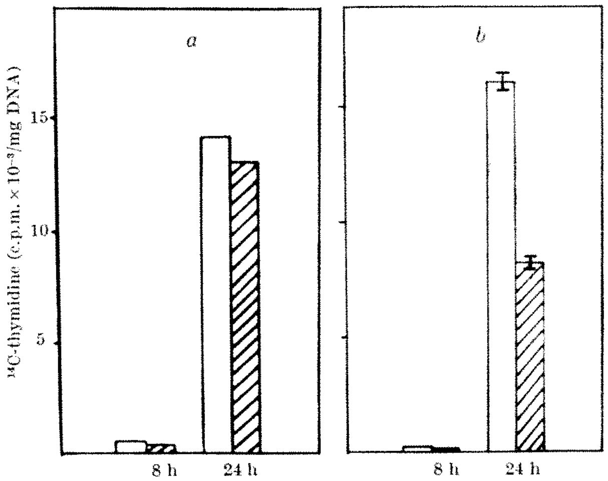

Fig. 1. Effects of GA and ABA on DNA synthesis. Wheat embryos were germinated in 3 cm sterilized Petri dishes on a 1 per cent agar medium containing 2 per cent sucrose and 10 µg/ml. chloramphenicol. Germination was started by adding to the embryos on the surface of the agar 0·6 ml. of 0·005 M *tris*-(hydroxymethyl)-aminomethane buffer (*p*H 7·6), 0·02 M KCl, 20 µg/ml. sucrose, 10 µg/ml. chloramphenicol (germinating medium, GM) or GM including either GA or ABA (1×10^{-4} M). The plates were then maintained in darkness at 25° C. After 7 and 23 h, they were transferred for 1 h to a corresponding medium containing 1 µCi/ml. ^{14}C-thymidine (Radiochemical Centre, Amersham; 56 mCi/mmole). Embryos were then homogenized in 5 ml. of 0·1 M EDTA–saline buffer (*p*H 8). DNA was isolated from the total homogenate, purified as previously described[7] and plated on to glass fibre filters ('GFA') and the radioactivity counted in a Beckman scintillation counter. Background values for the absorption of radioactivity were estimated in dry and germinated embryo samples homogenized in the presence of the radioactive precursor medium and processed as for the experimental samples. Open areas, controls. Hatched areas: *a*, 1×10^{-4} M GA; *b*, 1×10^{-4} M ABA.

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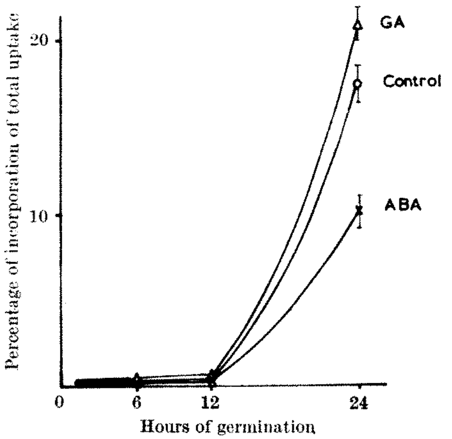

Fig. 2. Effect of GA and ABA on incorporation of uridine. Wheat embryos, 300 mg per sample, were germinated in the presence of GM or GM containing 1×10^{-4} M GA or ABA as described in Fig. 1. After 11 h the embryos were transferred to the respective radioactive media containing 15 μCi/ml. ^{3}H-uridine (Radiochemical Centre, Amersham; 19·2 Ci/mmole) for 1 h before analysis. The embryos were then homogenized in 5 ml. of 0·01 M Tris (*p*H 7·8), 0·02 M KCl, 0·01 M $MgCl_2$ (TM buffer) and 1 ml. aliquots were diluted with 5 ml. of 5 per cent TCA, filtered on 'GFA' glass fibre filters, washed sequentially with 10 ml. 5 per cent TCA, 3 ml. of ethanol, 3 ml. ether; then dried and counted. Aliquots (0·1 ml.) of the original homogenate were dried on 'GFA' filter discs and the radioactivity was counted for the estimation of total uptake of ^{3}H-uridine. Absorption backgrounds were estimated as in the legend to Fig. 1.

Protein Synthesis

Protein synthesis is initiated in the germinating wheat embryo immediately after imbibition[6] and evidence suggests that this early protein synthesis is programmed by a masked mRNA preserved in the dry embryo[5,7]. We now report the effects of GA and ABA on protein synthesis during the first 24 h of germination. Embryos were germinated in GA or ABA and pulsed with a ^{14}C-amino-acid mixture for 30 min before analysis. A stimulation of amino-acid incorporation by GA can be detected as early as 8 h, and by 24 h incorporation is 20–30 per cent higher than that in the controls (Fig. 3). With ABA, however, there was no detectable difference between treatments and controls for the first 6 h, but subsequently incorporation was inhibited by ABA (Fig. 4).

If the rate of protein synthesis in GA and ABA were a reflexion of the extent of polysome formation, this should be detectable in the distribution pattern of the isolated ribosomes. Ribosomes were therefore isolated[7] from embryos that were germinated for 12 h in germination medium alone, or in germination medium containing GA or ABA. They were layered on a sucrose gradient which was centrifuged and fractionated. The spectrophotometric analysis showed that the resulting absorbance patterns did not differ between the various treatments (Fig. 5*a*).

When ribosomes isolated from control 12 h embryos were incubated with either GA or ABA for 10 min at 20° C no differences were found in the subsequent distribution pattern of the ribosomal population, an effect reported before for ABA in leaf tissue[20]. But when embryos were pulsed with a ^{14}C-amino-acid mixture between 11 and 12 h of germination, the ribosomes fractionated from GA treatments—although exhibiting no difference in absorbance pattern compared with controls—displayed enhanced incorporation over the whole gradient. By contrast the ribosomes isolated from ABA treated embryos manifested reduced levels of amino-acid incorporation (Fig. 5*b*) compared with controls.

In other experiments, ribosomes isolated from 12 h embryos pretreated with GA or ABA were purified and tested for their endogenous mRNA activity in a cell free amino-acid incorporating system, saturated with tRNA and supernatant obtained from wheat germ. The results (Fig. 6) show that ribosomes from GA treated embryos display a 23–28 per cent higher template activity than controls, but ribosomes from ABA treated embryos have a slightly decreased activity (15–20 per cent) with respect to the equivalent amount of control ribosomes.

We have also tested whether the ribosomes *per se* are altered by the addition of the hormones *in vivo*. Stripped ribosomes from GA and ABA treated embryos were incubated in the standard cell free system together with poly U and ^{14}C-phenylalanine. Neither GA nor ABA treatment affected the capacity of the ribosomes to support peptide bond formation. The values for ^{14}C-phenylalanine incorporated by the three different ribosomal preparations in a typical experiment were 288,820, 276,100 and 275,610 c.p.m. per mg ribosomes.

The effect of a direct addition of GA or ABA to the cell free system primed by poly U has also been determined (legend to Fig. 6). ABA has a direct inhibitory effect on incorporation ranging from 10 per cent at $1·25 \times 10^{-5}$ M to 30 per cent at $2·5 \times 10^{-4}$ M. Similar effects are found with GA; however, at 10^{-5} M, inhibition was approximately 5 per cent and rose to 24 per cent at 2×10^{-4} M. This experiment rules out the possibility of a direct stimulating

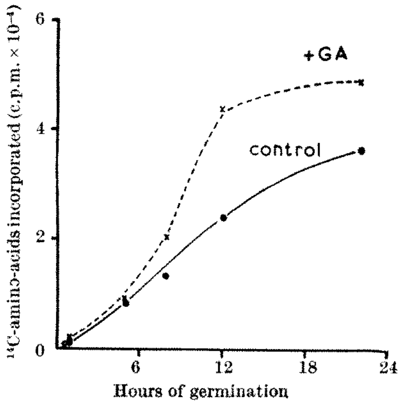

Fig. 3. Effects of GA on incorporation of amino-acids. Wheat embryos, 300 mg per sample, were germinated for different times before being transferred for 30 min into GM solutions containing 1 μCi/ml. ^{14}C-amino-acid mixture (New England Nuclear, 1·2 mCi/mg) with or without 1×10^{-4} M GA. The embryos were then washed with 30 ml. of GM containing 0·5 mg/ml. protein hydrolysate, homogenized in 8 ml. cold 5 per cent TCA, heated for 15 min at 90° C, and cooled; 0·5 ml. aliquots were filtered on sartorius membrane filters (0·45 μm), dried and the radioactivity was counted. Results are expressed as total counts incorporated into the embryo sample, but the pattern is similar if expressed as per cent incorporation of total uptake. Absorption backgrounds were estimated as in the legend to Fig. 1.

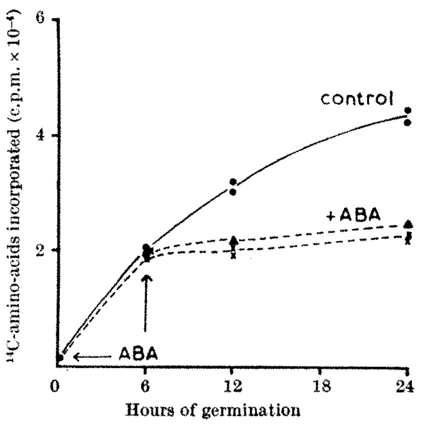

Fig. 4. Effect of ABA on protein synthesis. Wheat embryos, 300 mg per sample, were germinated in GM (●—●), or GM containing 2×10^{-4} M ABA (×---×). In one set of samples, embryos were germinated for 6 h in GM before being transferred to GM + ABA medium (▲---▲). At various intervals the embryos were transferred for 30 min to the respective medium containing 1 μCi/ml. ^{14}C-amino-acid mixture and processed; the radioactivity was counted, and the results are expressed as in the legend to Fig. 3.

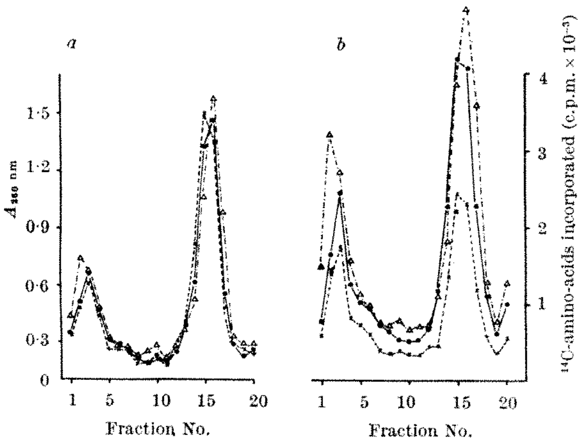

Fig. 5. *In vivo* effects of GA and ABA on distribution pattern (*a*) and activity (*b*) of ribosomes. Wheat embryos, 500 mg samples, were imbibed in GM or GM containing 1×10^{-4} M GA or ABA. After 11 h the germinating media contained 2 µCi/ml. ^{14}C-amino-acid mixture (New England Nuclear, 1·2 µCi/µg). Ribosomes were isolated from embryos after 12 h of germination by homogenizing them in 10 ml. of 0·05 M Tris (*p*H 7·8), 0·01 M $MgCl_2$, 0·025 M KCl, 0·5 M sucrose–0·005 M mercaptoethanol (R buffer) and centrifuging for 20 min at 12,000 r.p.m. The supernatant was then centrifuged for 2 h at 50,000 r.p.m. in the Spinco '65' rotor, the pellet dispersed in 10 ml. of R buffer and recentrifuged for 45 min at 50,000 r.p.m. The pellet containing the ribosomes was dispersed in 1 ml. of TM buffer, centrifuged for 10 min at 10,000 r.p.m., and 0·3 ml. aliquots were fractionated on 5–20 per cent sucrose gradient (Spinco 'SW 39' rotor, 36,000 r.p.m., 45 min). Gradient fractions were analysed spectrophotometrically at 260 nm, precipitated by 3 ml. cold 5 per cent TCA, heated for 15 min at 90° C, cooled, filtered on to sartorius membrane filters, washed, dried and counted. ●—●, Control; △—·—△, GA; ×---×, ABA.

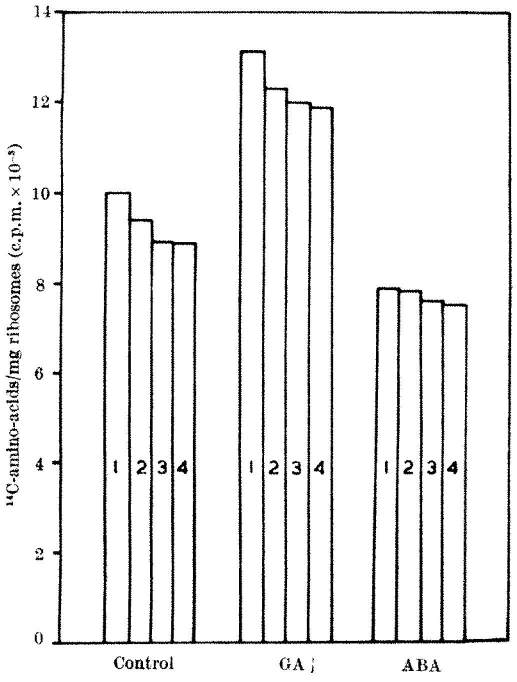

Fig. 6. Template activity of ribosomes from GA and ABA treated embryos. Wheat embryos, 1 g per sample, were germinated for 12 h in the presence of 1×10^{-4} M GA or ABA or GM only. Ribosomes were then isolated as described in Fig. 5 and the endogenous messenger activity was tested in a standard cell free system, containing (in a volume of 0·4 ml.) 22 mM Tris buffer (*p*H 7·8), 2·2 mM $MgCl_2$, 0·3 mM GTP, 0·4 mM ATP, 16 µg pyruvate kinase, 45 µg phosphoenolpyruvic acid, 6 mM mercaptoethanol, 50 µg tRNA (wheat), wheat germ 100,000*g* supernatant (0·5 mg protein), 100 µg ribosomes and 0·5 µCi ^{14}C-amino-acids (New England Nuclear, 1·2 mCi/mg). Incubation was at 25° C. The reaction was linear for 45 min but after 30 min the reaction was stopped with 3 ml. cold 5 per cent TCA. The precipitate was boiled for 15 min, cooled, filtered and counted. Background counts determined by adding ribosomes to the complete mixture were 200–300 c.p.m.

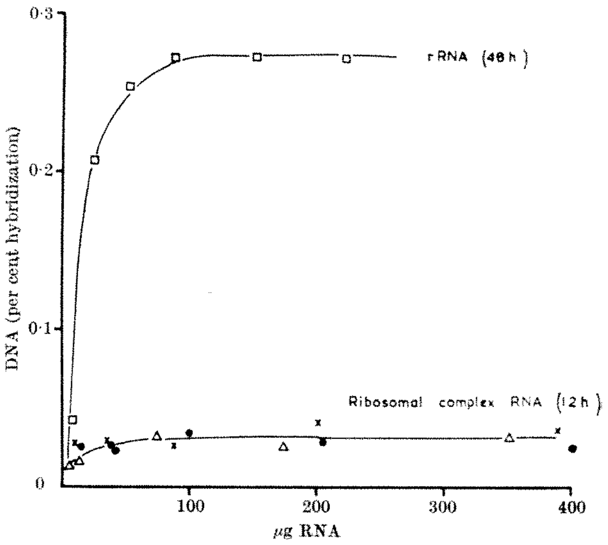

Fig. 7. Hybridization of DNA from 24 h germinated embryos with ^{3}H-RNA from the ribosomal complex. Embryos were germinated for 12 h in the presence of GA or ABA in media including 100 µCi/ml. ^{3}H-uridine. Ribosomes were isolated and their total RNA was extracted and extensively purified by two successive treatments with deoxyribonuclease followed by column chromatography on methylated albumin-kieselguhr, denaturation at 90° C for 3 min and filtration through nitrocellulose membrane filters. Increasing amounts of the RNA were annealed[5,22] with constant amounts of wheat embryo DNA (71 µg per filter). Purified rRNA was annealed as a control. The specificity of hybridization has been described[7]. ●, ^{3}H-RNA (519 c.p.m./µg) obtained from embryos germinated in GM; △, ^{3}H-RNA (620 c.p.m./µg) obtained from embryos germinated in GM containing 1×10^{-4} M GA; ×, ^{3}H-RNA (416 c.p.m./µg) obtained from embryos germinated in GM containing 2×10^{-4} M ABA; □, ^{32}P-rRNA (3,200 c.p.m./µg) from 48 h germinated embryos.

effect of GA on any of the transfer enzymes[21], but no conclusion can be drawn with regard to an effect on chain initiation or aminoacyl-tRNA synthetases (except for phenylalanine tRNA synthetase itself).

Enhanced activity of ribosomes from GA treated embryos might be expected if they were primed by newly transcribed message induced by the GA, a requirement of the concept of gene depression. Conversely, a reduction in newly transcribed message should be found for ABA. Active message that is newly synthesized should be present on the ribosomes and should therefore be detectable by molecular hybridization to the DNA. We have tested this model by measuring the percentage hybridization of RNA from the ribosome complex of 12 h embryos at saturation levels of the DNA (Fig. 7). The hybrid formed with the newly synthesized RNA accounts for no more than 0·03 per cent of the genome and no differences could be detected among the preparations from control and hormone treated embryos. Chased ribosomal RNA[7] from 48 h embryos was annealed as a check for total rRNA and hybridized to about 0·3 per cent of the DNA. We conclude therefore (*a*) that newly synthesized ribosomes are not yet present in the cytoplasm at 12 h of germination and (*b*) that the mRNA that is active on the ribosomes at 12 h is not newly transcribed. We cannot, however, exclude an effect on the transcription of a small and specific RNA fraction which is below the level of detection by the techniques we have used. Transfer RNA could be such a specific gene product responsible for the stimulation of protein synthesis although other experiments (unpublished) do not support this. We find no evidence that GA or ABA modifies either the levels or the charging of tRNAs during the first 12 h of germination.

Total protein synthesis in germinating wheat embryos can be stimulated *in vivo* by GA and the *in vivo* stimulation is stabilized in the cell free system; direct addition of GA to the cell free system is ineffective. These results suggest the requirement of a cofactor for GA function. This cofactor could be associated with the membranes.

nucleus or cell walls, or it could be readily dialysable, for it is not present in the supernatant, the tRNA or the ribosomal components of the cell free system. Alternatively, it could be highly labile once cells are disrupted.

Hormonal substances evoke their greatest responses when applied to intact cells, although some success in demonstrating regulatory control in isolated organelles has been achieved if the hormone is present during the isolation procedure[23]. Few examples of activity are known for hormones added directly to isolated organelles or to isolated chromatin. In these instances either some fraction of the cytoplasm or a specific protein(s) seems to mediate the activity of the hormone molecules[24-27].

The fact that neither GA nor ABA affects the pattern of monosome and polysome distribution (Fig. 5), while endogenous template activity of the ribosomal fractions from hormone treated embryos is altered (Fig. 6), points to an action of the hormones at the level of messenger availability. The hybridization experiments (Fig. 7), which show that no new mRNA or rRNA is associated with the active ribosomes, indicate that the hormones regulate the use of mRNA already stored in the embryo rather than controlling mRNA synthesis *de novo*.

We conclude that whatever effects the hormones may have on transcription, they are secondary to control at the level of translation, and that, provided the cofactor(s) are available, GA can function in the germination of the wheat embryo by controlling the expression of preformed messages. This could be achieved by unmasking informosomes[28], facilitating the interaction of message with the ribosomes or by regulating the rate of message read out. The action of ABA seems also to be at the translation level, but may be at sites different from those of GA.

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Contribution of the GABA Bypath to Glucose Oxidation, and the Development of Compartmentation in the Brain

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Post mortem changes in GABA provide a means of assessing the normal GABA flux *in vivo*. Compartmentation involving glutamate and GABA develops with the formation of nerve terminals during the maturation of the brain.

WHEN glucose is oxidized in the brain, a part of the glucose carbon is oxidized in the GABA bypath. Earlier estimates of the magnitude of the GABA flux ranged from 10–40 per cent of the total flux through the tricarboxylic acid cycle, but the methods used were subject to error because of compartmentation or other factors. A more recent estimate obtained by following the oxidation of glucose in brain slices prelabelled with [1-^{14}C]GABA gave the flux through the GABA bypath as 8 per cent of the flux through the tricarboxylic acid cycle[1,2]. GABA formation continues for an appreciable time after death and newly synthesized GABA accumulates in the brain[3]. Assuming the initial rate of GABA formation post mortem is similar to the normal rate *in vivo*, this could offer an independent method of obtaining evidence of the normal GABA flux *in vivo*.

GABA Flux

The post mortem changes in the concentration of GABA and glutamate are shown in Fig. 1*a*. The fractional increase of about 30 per cent at 4 min after death was too small to affect the amount of glutamate in the tissue. The GABA flux *in vivo* was estimated by making assumptions similar to those made by Lowry *et al.*[6] in calculating the *in vivo* rate of energy metabolism in the brain from post mortem changes in metabolite concentrations: first, that reactions requiring O_2 stopped immediately on the arrest of the circulation and, second, that GABA formation proceeded initially at the same rate post mortem as *in vivo*. The GABA flux calculated on this basis, by fitting a regression line to the data, was $0{\cdot}127 \pm 0{\cdot}0196$ (s.e.) μmoles/g/min. The total flux through the tricarboxylic acid cycle may be estimated from the data of Lowry *et al.*[6] and Gaitonde[7] by assuming that 15 per cent of the glucose used was converted into lactate[8] and that the remainder yielded 2 moles of acetyl-CoA per mole of glucose, which were oxidized to CO_2 in the tricarboxylic acid cycle. The flux in the tricarboxylic acid cycle according to these calculations is 1·3–1·6 μmoles/g wet weight/min; the GABA flux was therefore 8–10 per cent of the total flux in the cycle.

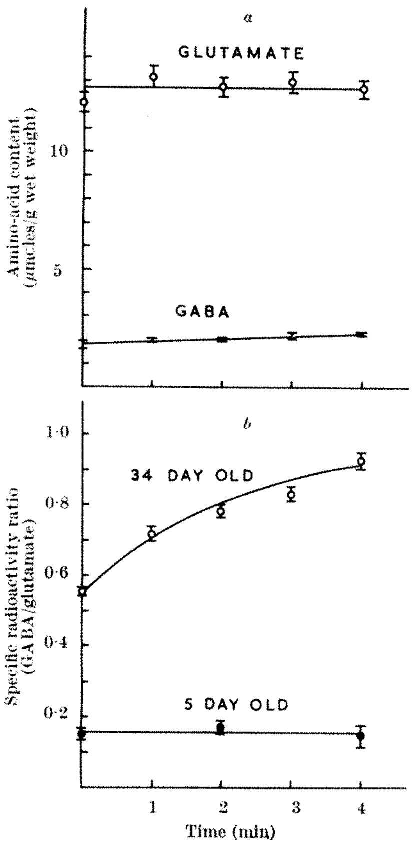

Fig. 1. *a*, Post mortem increase in the content of GABA in the brain of 34 day old albino mice (μmoles/g wet weight). The data were analysed by analysis of variance; the means ± s.e. were plotted (the total number of separate observations was nineteen). Regression lines were fitted to the data. The equation of the line representing the content of GABA per unit weight was $y = 0{\cdot}127x + 1{\cdot}816$; regression coefficient $= 0{\cdot}127 \pm 0{\cdot}0196$ ($P < 0{\cdot}001$). The slope of the regression line for glutamate content was not significantly different from zero: similarly from analysis of variance the values for glutamate content at different times were not significantly different. *b*, Post mortem change in the GABA/glutamate specific radioactivity ratio. The data were analysed and plotted as in *a*; total numbers of individual observations were nine and nineteen for the 5 and 34 day old mice, respectively.

Mice (LAC Carshalton), 5 and 34 days old, received a subcutaneous injection of [2-^{14}C] glucose (52·4 mCi/mmole; dose 20 μCi/100 g body weight). At 10 min after injection one group of mice were killed by immersion into liquid N_2 ("0" time). The other animals were decapitated at the times indicated, brains were quickly excised and the two cerebral hemispheres kept in a humid chamber at 37° C (ref. 4). Three hemispheres of 5 day old mice were pooled for each time-point; one hemisphere from each animal corresponds to the 2 min and the other to the 4 min point. Each time-point in (*b*) is the mean for three sets of pooled tissues. The two hemispheres of the 34 day old mice provided two time-points: each was processed individually. The total numbers of hemispheres used were four at 2 and 4 min, and three at 1 and 3 min. Five whole brains were used for the estimations at "0" time. The metabolic reactions were stopped by freezing in liquid N_2. For the analysis of GABA and glutamate, the brains were extracted with 0·4 M $HClO_4$ and the methods used were similar to those of A. J. P. and R. B. Neutral compounds and carboxylic acids were removed from the acid soluble extracts by a cation exchange resin (Dowex 50, H^+ form). Acidic amino-acids and glutamine were separated by an anion exchange resin (Dowex 1, acetate form). The remaining amino-acids were then adsorbed on a column of Dowex 50 (H^+ form) resin and the neutral amino-acids eluted with 1 M pyridine, leaving the basic amino-acids and ethanolamine on the resin. The neutral amino-acids were processed as described[1]. GABA was separated by paper chromatography (Whatman No. 1; 40 h) using butan-l-ol–propionic acid–water (10 : 5 : 7) and marker strips. The GABA was eluted, a fraction used for determining radioactivity by scintillation spectrometry and another fraction for estimating the α-amino-N content with a Technicon autoanalyser after desiccation.

The absolute value of the GABA flux was higher than that previously estimated using guinea-pig cerebral cortex slices *in vitro*[1,2]; but the relative value was approximately the same as determined before—about 8 per cent of the total flux.

Compartmentation of GABA Formation

A model consisting of a minimum of three compartments is consistent with the data hitherto obtained on the labelling of glutamate and glutamine in brain tissue[1]. Each "compartment" probably results from the summation of a number of different units of more or less similar metabolic pattern. The combination constituting what is defined on a functional basis as a "small" or a "large" compartment[9-11], in view of the size of the related glutamate pool, will depend on the site of entry and metabolism of the substrate considered[1]. The "small" compartment can oxidize a wide range of substrates, including short chain fatty acids and amino-acids, whereas glucose is the main substrate in the "large" compartment[5,9-13]. The "large" compartment has a "sub-compartment" containing glutamate which is apparently the precursor of the "synaptic" pool of GABA (refs. 1, 14 and personal communication from D. Garfinkel).

In these present experiments on 34 day old mice the specific radioactivity of GABA at 10 min after injection of [2-^{14}C]glucose was about half of that of glutamate (ratio 0·55 ± 0·013 [5] at "0" time in Fig. 1*b*). In agreement with Minard and Mushahwar[15], the GABA/glutamate specific radioactivity ratio increased post mortem and at 4 min after death it was almost unity (0·93 ± 0·015[4]) (Fig. 1*b*). Because each experimental animal provided two post mortem time points it was possible to calculate with reasonable accuracy the specific radioactivity of the glutamate from which GABA was formed during the different time intervals after death. The mean specific radioactivity of this glutamate was 54 ± 5 per cent [7] higher than that of the total glutamate in the tissue, confirming the heterogeneity of the "large" glutamate pool.

Development of Compartmentation of GABA Formation

Quantitative morphological studies have shown that the phase of rapid formation of nerve terminals in rat brain occurs during the period from 12 to 26 days after birth[16]. The formation of nerve terminals coincides with the development of metabolic compartmentation in this species[5]. We therefore investigated whether or not the glutamate pool associated with the "synaptic" pool of GABA develops during the maturation of the mouse brain. After administration of [2-^{14}C]glucose the GABA/glutamate specific radioactivity ratio was much lower in the immature 5 day old brain than in the "adult" brain ("0" time in Fig. 1*b*; 0·15 ± 0·016 [3]). By contrast with the results obtained in the "adult" brain, the ratio did not increase during the 4 min experimental period. Thus the glutamate pool, which is the precursor of the GABA formed post mortem, seems to develop at about the time of rapid formation of nerve terminals during the maturation of the brain.

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LETTERS TO THE EDITOR

BIOLOGICAL SCIENCES

Anti-poxvirus Activity of Rifampicin associated with Hydrazone Side Chain

RIFAMPICIN[1] inhibits the replication of vaccinia virus in infected cells *in vitro* when added at 100 µg/ml. immediately after infection[3,4] or at a late stage of the virus latent period[4]. In the presence of the drug the translation of late viral mRNA species was found to be markedly inhibited 8 h after infection[5]; the rate of late viral protein synthesis declined[6]; the synthesis of the particulate viral RNA polymerase molecules, induced late in infection, was prevented[7]; and the assembly of virions was affected[8,9]. Rifampicin did not affect the synthesis of viral mRNA molecules nor their association with ribosomes to form polyribosomes[5]. The inability of rifampicin to inhibit *in vitro* the activity of the three different species of poxvirus specific DNA dependent RNA polymerases[7] suggested that the mode of antiviral activity of rifampicin differed from its antimicrobial activity. The latter was attributed[10] to the macrocyclic ring of the rifamycin SV molecule which binds to the microbial DNA dependent RNA polymerase and inhibits the initiation of RNA synthesis. Using Shope fibroma virus (SFV), a poxvirus which causes localized tumours in rabbits[11-13], and several rifamycin SV derivatives, we have obtained results which indicate that the anti-poxvirus activity resides in the hydrazone side chain of rifampicin.

Table 1. THE STRUCTURE OF THE SIDE CHAINS AT CARBON 3 OF RIFAMYCIN SV AND THEIR RELATIVE ANTI-POXVIRUS ACTIVITY

Name of compound	Compound No.*	Structure of side chain at position 3	Effect on SFV replication
Rifampicin	31	$-CH=N-\underbrace{N(CH_2)_2N(CH_3)CH_2CH_2}$	Inhibition
—	18	$-CH=N-N(CH_3)_2$	Inhibition
—	11	$-CH=N-C_3H_2NS$	Partial inhibition
—	33	$-CH=N-N(CH_3)CH_2CH_2N(CH_3)_2$	No effect
—	L 8141	$=N-NH\overset{\overset{S}{\|}}{C}NH_2$	No effect
Rifamycin SV	—	$-H$	No effect

* The compound numbers are those of ref. 2.

Our strain of SFV was propagated and assayed first in primary rabbit kidney cell cultures and later in BSC_1 cell cultures and retained the ability to cause tumours in rabbits. The different rifamycin SV derivatives (Table 1), at various concentrations, were added to BSC_1 cell cultures immediately after infection with a low (200–300 p.f.u./plate), or high (10 p.f.u./cell) virus multiplicity, and incubated at 37° C. Isatin-β-thiosemicarbazone (IBT), a potent anti-poxvirus inhibitor[14], was used to inhibit SFV infection at a concentration of 5 µg/ml. The effect of the rifamycin SV derivatives on the development of SFV plaques in infected BSC_1 cell monlayers was studied by adding different concentrations of drug to monolayer cultures which were infected with about 200 p.f.u./plate. The cultures were reincubated for 2 days at 37° C and the plaques in each plate were counted. Table 2 shows that 80 µg/ml. of rifampicin inhibited the development of SFV plaques[4,5]. Compounds 11 and 18 also inhibited the development of SFV plaques but at a concentration of 120 µg/ml., but compound 33, rifamide, rifamycin SV (Table 2), and a thiosemicarbazone derivative (L 8141) of formylrifamycin (recently synthesized by Professor P. Sensi, Gruppo Lepetit, Milan) had no effect on the development of SFV plaques even at 150 µg/ml. On the other hand, IBT (ref. 12) completely inhibited the formation of SFV plaques at a concentration of 5 µg/ml.

Table 2. THE EFFECT OF RIFAMPICIN AND DERIVATIVES ON THE DEVELOPMENT OF SFV

Drug concentration (µg/ml.)	No. of plaques per plate* Drug: 31	11	18	33
0	186	154	154	154
50	131	114	113	145
60	126	183	162	144
70	33	109	142	125
80	0	73	116	146
90	0	—	—	—
100	0	4	12	154
120	0	0	0	177

* Average of six experiments.

The effect of the rifamycin SV and its derivative rifamide on the growth cycle of SFV was compared with that of rifampicin and IBT. Cell cultures were infected with SFV at high multiplicity and immediately treated with the different drugs. We found that synthesis of the new SFV progeny started in the infected cells 12 h after infection and the number of infectious virions gradually increased until 30 h after infection. Addition of rifampicin immediately after infection inhibited the synthesis of SFV, and the amount of the residual virus gradually decreased possibly because of continuation of the uncoating processes (Fig. 1). By contrast with rifampicin, which completely inhibited the replication of SFV and prevented the development of virus progeny, rifamide and rifamycin SV had no effect on the initial steps of the virus replication, but caused a reduction of the final yield of virus. The nature of this effect is not yet clear. Isatin-β-thiosemicarbazone effectively inhibited the synthesis of infectious virions at a level of 5 µg/ml. in contrast to 100 µg/ml. of rifampicin (Fig. 1). Nevertheless, a newly synthesized derivative (L 8141), which had a thiosemicarbazone side chain at carbon 3 instead of the hydrazone molecule, had no effect on the replication of SFV or vaccinia virus. To assess the importance of the hydrazone side chain for the

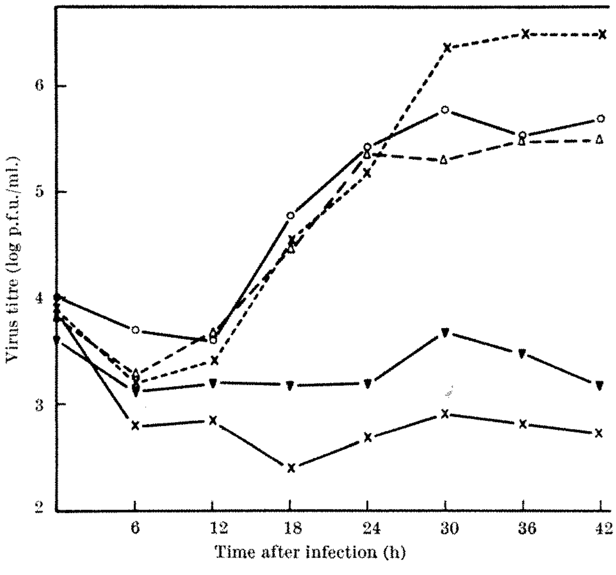

Fig. 1. The effect of rifampicin, rifamycin SV, rifamide and IBT on the replication of SFV. Cell cultures were infected with SFV (10 p.f.u./cell). Part of the cultures was untreated (× - - - ×); the others were treated with rifampicin (100 µg/ml., × — ×); rifamycin SV (100 µg/ml., △ - - - △); rifamide (100 µg/ml., ○—○); or IBT (▼—▼). Samples were removed at different times after infection and the virus yield was determined.

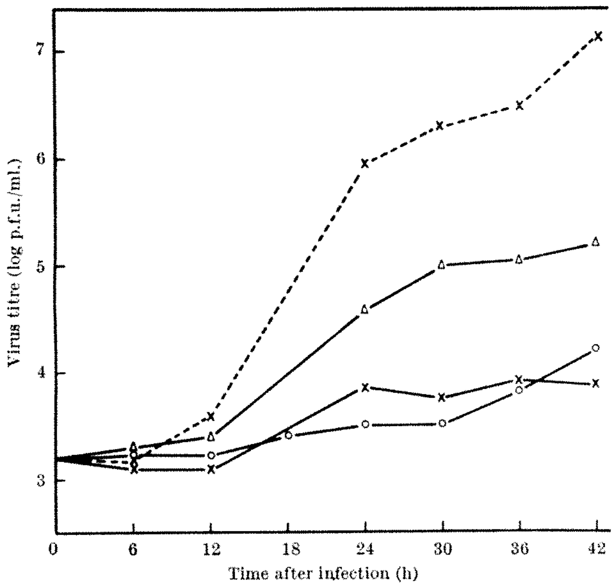

Fig. 2. The effect of compounds 31 (rifampicin), 18 and 11 on the replication of SFV. Cultures were infected with SFV and treated with rifampicin (compound 31, × — ×), compound 18 (○—○), or compound 11 (△—△), each at a concentration of 100 μg/ml. Infected untreated cultures (× - - - ×) were used as controls. The virus yield at different time intervals after infection was determined.

anti-poxvirus activity of rifampicin, the effect of compounds 11 and 18 (Table 1) on the growth cycle of SFV was studied. Compound 33 was not tested because it had no effect on the development of SFV plaques (Table 2). The drugs (100 μg/ml.) were added to the infected cultures immediately after infection. Fig. 2 shows that compound 18 completely inhibited the replication of SFV, as effectively as rifampicin, whereas compound 11 reduced but did not prevent virus replication. Thus compound 18 is a new effective anti-poxvirus derivative of rifamycin SV: both rifampicin and compound 18 were found to inhibit the synthesis of the poxvirus progeny while present in the cells. Washing of the infected, rifampicin treated cells restored the synthesis of SFV virions to a rate similar to that in the infected untreated cultures. Most of the virus progeny was synthesized within the first 24 h after the removal of rifampicin.

These results demonstrate that rifampicin, at a concentration of 100 μg/ml., prevents the replication of SFV in infected cells; SFV resembles vaccinia virus in its sensitivity to rifampicin[3,4]; the inhibitory effect of the drugs persists only as long as they are present in the infected cells; and the hydrazone side chain of rifampicin is responsible for the anti-poxvirus activity of the drug. Removal of the hydrazone side chain from the rifampicin molecule, its replacement by thiosemicarbazone or modifications in the hydrazone side chain causes loss of the antiviral activity. Compound 18, which was found to inhibit SFV, had a low anti-trachoma activity[15,16], whereas compound 33, which lacked anti-poxvirus activity, was found to be a potent anti-trachoma drug[17]. Both rifampicin and compound 18 were found to be less effective in inhibiting SFV than IBT, which is known to affect the binding of late viral mRNA molecules to ribosomes in cells infected with vaccinia virus[18]. A thiosemicarbazone side chain at carbon 3 of rifamycin SV, however, had no effect on the replication of SFV and vaccinia virus. The molecular mechanism by which the hydrazone side chains of rifampicin and compound 18 interfere with the replication of poxviruses is still to be elucidated.

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Partial Failure of Methylation and Cleavage of 45S RNA in the Blast Cells of Acute Leukaemia

IN animal cells the synthesis of ribosomal RNA follows a definite molecular pattern. The two ribosomal RNA subunits, 28S and 18S RNA, are synthesized in the nucleolus as a large 45S molecule[1,2]. The latter is rapidly cleaved into an 18S molecule and into a 32S molecule. The 18S molecule reaches the cytoplasm immediately. The 32S molecule is converted into a 28S molecule in the nucleolus before reaching the cytoplasm[3].

One of the principal chemical events occurring during this process is the attachment of methyl groups to the 2′ OH of the ribose of ribosomal RNA. The 45S RNA is the main if not the only site of methylation[4,5] so that by studying the distribution of labelled methyl groups in the different types of RNA molecules it is possible to follow the stepwise formation of mature ribosomal RNA. While all the methyl groups incorporated in 45S RNA are preserved during further RNA processing[6], about half of the 45S molecule is lost as RNA maturation proceeds (refs. 6–8).

In rapidly growing cells, such as HeLa cells or phytohaemagglutinin stimulated lymphocytes, the entire process occurs rapidly: the average life of the 45S molecule in HeLa cells is about 15 min. After growing animal cells have been incubated with labelled RNA precursors, the label is found associated with 45S RNA only after short periods of exposure (5–15 min); longer periods bring about an extensive labelling of 28S and 18S RNA.

We have attempted to study the processing of rapidly labelled RNA in blast cells of acute leukaemia, by investigating the sedimentation characteristics of labelled RNA after different periods of exposure to 5-^{3}H-uridine and [^{3}H-*methyl*]-methionine. Homogeneous blast cell populations were obtained from six untreated patients with leucocyte levels ranging from 35,000 to 90,000 per mm^3. Two of these patients had acute lymphoblastic leukaemia; one had acute monocytic leukaemia; three had acute myeloblastic leukaemia. The heparinized blood was

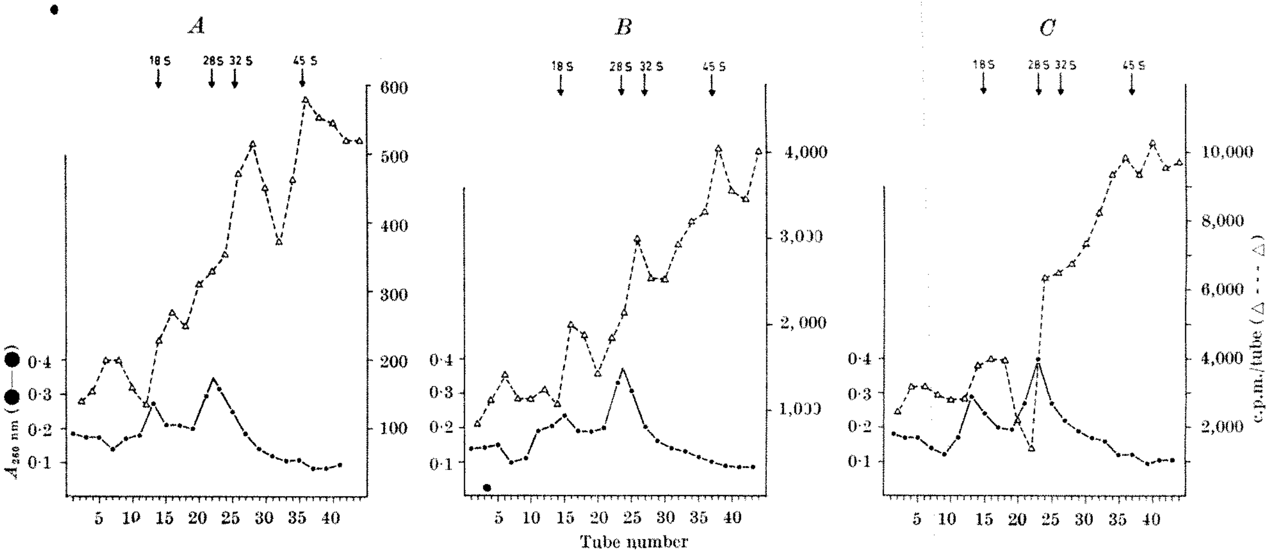

Fig. 1. Sedimentation pattern of labelled RNA extracted from circulating blast cells of patient 1, incubated with 5-^{3}H-uridine (10 µCi/ml.) for 30 min (*A*), 60 min (*B*) and 4 h (*C*). Because labelled blast cell RNA and unlabelled KB cell RNA were extracted simultaneously, the absorbance profile in this and in the following figures represents mainly the unlabelled KB RNA. Gradients were centrifuged at 16,000 r.p.m. for 16 h at 4° C. Approximate S values are indicated.

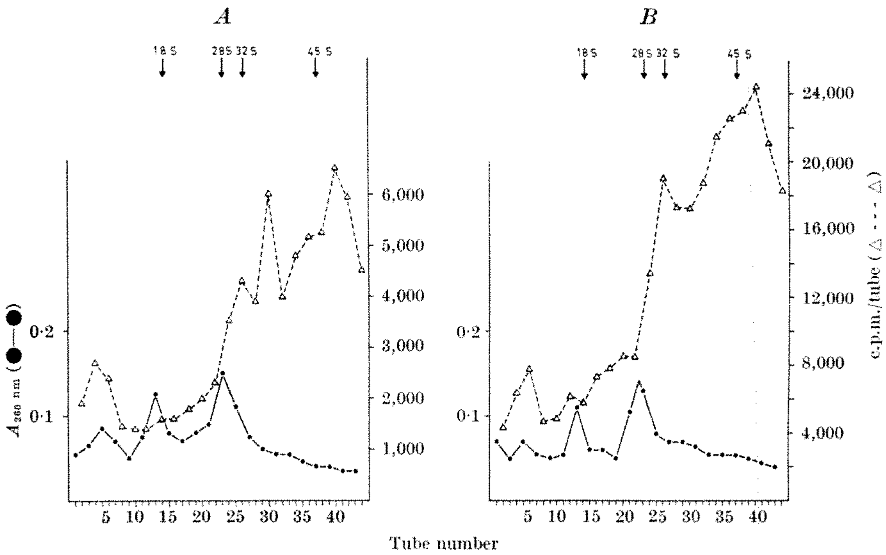

Fig. 2. Sedimentation pattern of labelled RNA extracted from circulating blast cells of patient 2, incubated with 5-^{3}H-uridine (10 µCi/ml.) for 2 h (*A*) and 6 h (*B*). Gradients were centrifuged at 16,000 r.p.m. for 16 h at 4° C.

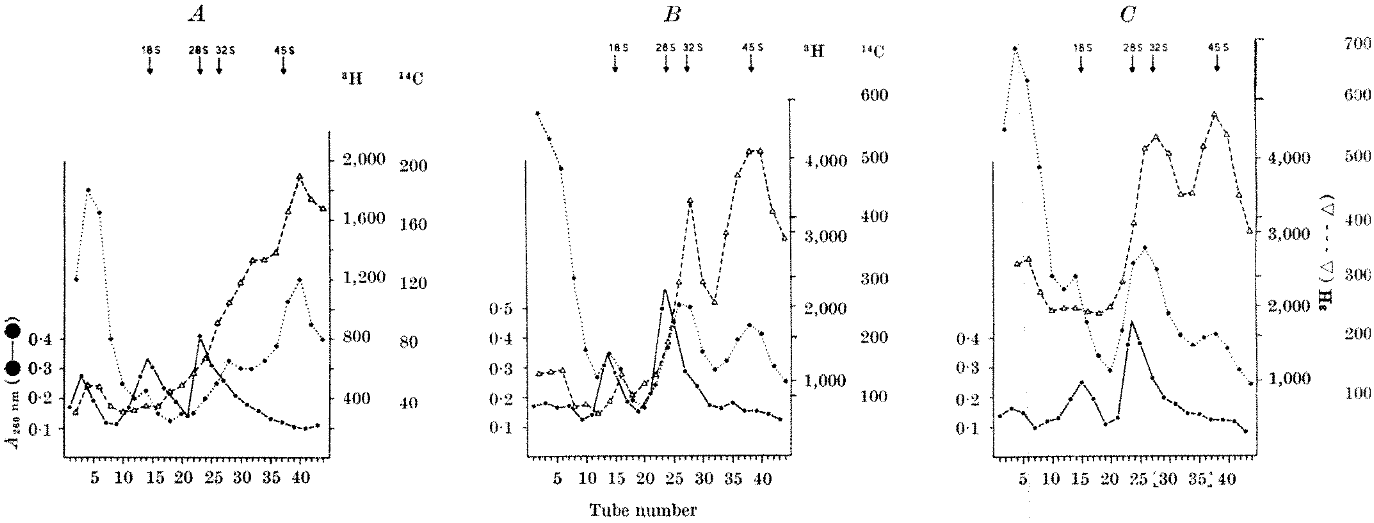

Fig. 3.* Sedimentation pattern of labelled RNA extracted from circulating blast cells of patient 4, incubated simultaneously with 5-^{3}H-uridine (5 µCi/ml.) and [^{14}C-*methyl*]-methionine (5 µCi/ml.) for 1 h (*A*), 3 h (*B*) and 5 h (*C*). Gradients were centrifuged at 16,000 r.p.m. for 16 h at 4° C.

sedimented under gravity, and the leucocyte layer was separated and diluted with minimal essential medium (MEM) and autologous plasma in order to obtain a cell concentration of $5{\cdot}0 \times 10^6$–$8{\cdot}0 \times 10^6$ per ml. with 20 per cent autologous plasma. Cultures (10 ml. each) were set up in rubber stoppered test tubes maintained at 37° C in a rotating system. After periods of incubation with the labelled precursors varying from 30 min to 6 h, the cells were harvested by chilling on frozen saline and centrifuged at 1,200 r.p.m. They were washed once with ice-cold saline. RNA was extracted by a modification[9] of the methods of Scherrer and Darnell[10] and of Kirby[11]. Because of the low number of blast cells used in each experiment, a suitable amount of unlabelled KB cell RNA was extracted simultaneously to act as a carrier. A quantity of RNA (15–30 *A* units) was centrifuged on 5–20 per cent linear sucrose gradients in 0·01 M Tris buffer, *p*H 7·4, at 16,000 r.p.m. for 16 h at 4° C in an '*SW* 25·1' rotor of a Spinco model *L* ultracentrifuge. Forty-five fractions were obtained with a gradient analyser (Isco), and absorption and radioactivity were assayed on alternate fractions[9]. The rate of 5-^{3}H-uridine incorporation was also studied by pipetting at 1 h intervals 200 µl. samples of the cultures in 50 volumes of 5 per cent trichloroacetic acid at 4° C. The precipitate was assayed for RNA radioactivity by a modification[9] of the Schneider[12] procedure.

In all cases studied, irrespective of the cytological type, the results were quite uniform. Incorporation of 5-^{3}H-uridine in acid-insoluble material gave a linear increase up to 7–8 h and decreased slowly afterwards. After short incubation periods (30–60 min) with 5-^{3}H-uridine, most of the radioactivity was associated with RNA with S values higher than 28 and with two main peaks at 32S–35S and 45S–50S (Fig. 1). Furthermore, in most cases, after 60 min incubation, a definite amount of radioactivity was associated with 18S RNA (Fig. 1). These findings indicated that the blast cells were synthesizing and processing ribosomal precursor RNA in a normal fashion, though at a somewhat slower rate. Normal behaviour of ribosomal RNA production would imply that, after longer periods of incubation, most of the radioactive label would be associated with 18S and 28S RNA, and the absorbance and radioactivity profiles would be superimposable. In no case could this be observed. On the contrary, exposure to 5-^{3}H-uridine up to 6 h resulted in an accumulation of radioactivity in the 30S–50S region of the gradient, the peak in 45S becoming extremely prominent (Figs. 1 and 2).

In a study of the distribution of RNA radioactivity after incubation with [^{3}H-*methyl*]-methionine definite peaks of radioactivity were observed at 45S, 32S–28S and 18S, closely corresponding to the peaks observed in the ^{3}H-uridine experiments, thus confirming that synthesis of ribosomal RNA took place in the cells studied. Contrary to the findings in the 5-^{3}H-uridine experiments, it was possible by prolongation of the incubation period to observe a gradual increase in radioactivity in the 32S–28S and 18S peaks, and a proportional decrease in the amount of label associated with the 45S RNA fraction.

We tentatively suggest that in the blast cells of acute leukaemia undermethylated 45S and 32S RNAs accumulate: a substantial amount of the former fails to undergo methylation and cleavage at a normal rate. This conclusion is supported by double-labelling experiments in which blast cells from three of our patients were incubated simultaneously with 5-^{3}H-uridine and [^{14}C-*methyl*]-methionine, and the two isotopes were evaluated in the same RNA fraction. It was observed that the ^{3}H-pyrimidine : ^{14}C-methyl ratio in 45S RNA was increased by prolonging the period of incubation (Fig. 3), thus indicating the accumulation of unmethylated unprocessed RNA molecules.

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Comparison of the Effects of LSD, Heliotrine and X-irradiation on Chromosome Breakage, and the Effects of LSD on the Rate of Cell Division

Cohen, Marinello and Back[1] (1967) first reported that lysergic acid diethylamide (LSD) increased chromosome abnormalities in human leucocytes. Although some later work failed to support this observation[2–4], others have confirmed that LSD damages chromosomes[5–10]. I have investigated the dose–effect relationship of LSD and the type of damage produced in chromosomes of leucocyte cultures of the marsupial *Potorous tridactylus* (rat-kangaroo), which has a few large chromosomes ($2\,n = 12$ in ♀ and 13 in ♂) that make scoring of damage relatively easy and accurate.

I compared this damage with that caused by X-irradiation in the same cell system, and also by heliotrine, a pyrrolizidine alkaloid which is a dangerous hepatotoxin and chemical mutagen[11–14] which damages chromosomes in plants[15,16] and animals[17].

Methods for setting up cultures and assessing cell division have been described before[17]. Fully oxygenated blood was needed for irradiation experiments, and so, to maintain comparable conditions, blood for all experiments was first oxygenated for 30 min[17]. Whole blood was then irradiated (22° C) with 25 r./min (HVL, 0·5 mm Cu; 235 kV peak).

LSD ('Dylisid', Sandoz) was added when the cultures were set up. The smallest dose (0·4 µg/ml.) was chosen on the assumption that 2,000 µg (among the larger doses taken by LSD users) was completely absorbed into the blood stream.

Table 1 shows details of chromosome breakage produced by different concentrations of LSD. All cultures received colcemid at the 72nd hour and were terminated at the 74th hour, except those which received 6·64 µg/ml. of LSD. Because the suppression of cell division at this concentration precluded an adequate estimation of chromosome damage at the 74th hour, treatment with colcemid was prolonged to the 93rd or 96th hour.

Chromatid and isochromatid deletions increased with the size of the dose. All chromosome exchanges observed were of the chromatid type: chromatid dicentrics, triradials and chromatid intrachanges. With the larger

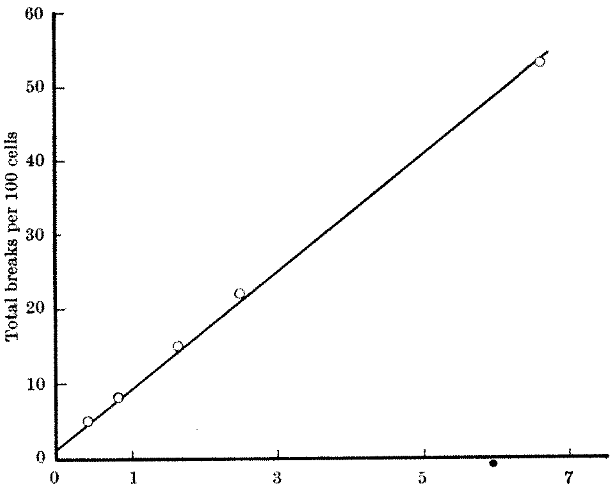

Fig. 1. Effect of LSD on chromosome breakage in leucocyte cultures of *Potorous tridactylus*. Aberrations due to culture conditions have been allowed for.

concentrations of LSD, there was an increase in elongation and attenuation of chromosome material, causing overlapping and difficulties in scoring some of the more damaged cells. This probably led to an underestimate of chromosome damage for the highest concentration of LSD (6·64 μg/ml.). For the range of concentrations used, a linear response was revealed when concentration was plotted against total chromosome breakage (Fig. 1).

Table 1. CHROMOSOME DAMAGE PRODUCED BY LSD IN LEUCOCYTE CULTURES FROM *Potorous tridactylus*

LSD (μg/ml.)	Abnormal nuclei (per cent)	Chromatid deletions (per cent)	Isochromatid deletions (per cent)	Chromatid exchanges (per cent)	Total breaks per cell	Cells scored
0	12	5	9	0	0·14	100
0·145	16	5	12	1·0	0·19	100
0·83	19	9	10	1·5	0·22	200
1·66	25	10	17	1·0	0·29	300
0	13	6	10	0	0·16	100
2·55	30	14	20	2·0	0·38	50
0*	24	15	16	1·0	0·33	100
6·64†	47	31	27	14·0	0·86	100
6·64*	52	39	27	12·0	0·90	100

Final concentration of colcemid was 0·004 per cent.

All cultures except with LSD concentration of 6·64 μg/ml. and the parallel control had colcemid treatment from the 72nd to the 74th hour.

* Cell division arrested from the 72nd to the 96th hour.

† Cell division arrested from the 72nd to the 93rd hour.

Because peripheral lymphocytes usually divide only when stimulated by phytohaemagglutinin, and because LSD was introduced when the cultures were set up, the drug must have been present for the whole cell cycle. Because only chromatid type exchanges were seen, LSD must act on chromosomes only during the period of DNA synthesis (S) or during the post-DNA synthetic period (G_2) when the chromosomes are already double structures. This means that chromosome damage in LSD users cannot be estimated from their peripheral leucocytes, because normally these cells *in vivo* are never at the appropriate stage. The actively dividing stem cells of the lymphoid tissue would be affected, but the more damaged cells would probably not survive to enter the blood stream. Furthermore, because the replenishment of the blood lymphocytes is dependent on many factors, there is no accurate way of determining the numbers of the less damaged cells or the time at which they enter the general circulation after administration of the drug.

Table 2 compares the total chromosome damage caused by LSD, heliotrine and X-irradiation. The total damage done by LSD was of the same order as that produced by heliotrine (1·66 μg/ml. of LSD produced 0·15 breaks/cell; 1·56 μg/ml. of heliotrine resulted in 0·10 breaks/cell). The high concentrations of LSD found in the liver[18] indicate that in habitual users of LSD, liver cells are continuously subjected to the effects of the drug and its breakdown products, for it is eliminated chiefly through this organ. In this connexion it is interesting that heliotrine causes liver lesions, and related pyrrolizidine alkaloids are known to be potent liver carcinogens[19].

Table 2. COMPARISON OF CHROMOSOME BREAKAGE PRODUCED BY LSD, HELIOTRINE AND X-IRRADIATION IN CULTURES OF LEUCOCYTES FROM *Potorous tridactylus*

Treatment	Total breaks/cell	Time of cell arrest (h)	Cells scored
μg/ml. of LSD			
0·415	0·05	72–74	100
0·83	0·08	72–74	200
1·66	0·15	72–74	300
2·50	0·22	72–74	50
6·64	0·53	72–96	100
6·64	0·57	72–93	100
μg of heliotrine*			
1·56	0·10	72–74	100
3·13	0·29	72–74	100
7·80	0·52	72–74	100
15·65	0·95	72–74	100
31·30	1·52	72–74	100
25 r./min of X-irradiation			
100 r.	0·36	72–74	100
200 r.	0·64	72–74	200
300 r.	0·92	72–74	100

* Results for heliotrine damage from ref. 17.

This table takes account of spontaneous aberration due to culture conditions.

LSD resembles heliotrine in preferentially attacking the heterochromatic regions of the chromosomes. With heliotrine, up to 30 per cent of the damaging events occurred in heterochromatin, but for LSD the figure was up to 50 per cent. By contrast, after X-irradiation only 5 per cent to 7 per cent of the total damage occurred in heterochromatin.

It is not possible to equate damage produced by a compound present for the whole of the culture period with that produced by an X-ray dose acting on the chromosomes only at the stage of the cell cycle at which it is delivered. But, as far as comparison is possible, a dose of 100 r. (at 25 r./min) produced a corresponding amount of damage as 4·2 μg/ml. of LSD (Fig. 1 and Table 2). A dose of 100 r. produced 0·0036 breaks/cell/r.[17]. A unit increase of 1 μg of LSD/ml. resulted in 0·078 breaks/cell.

Table 3. EFFECT OF LSD ON RATE OF CELL DIVISION IN LEUCOCYTE CULTURES FROM *Potorous tridactylus*

	Mitotic index as percentage of control				
	Time of arrest of cell division after initiation of cultures (h)				
μg/ml. of LSD	48–51	26–55	72–74	72–93	72–96
0·415	—	41	97	—	—
0·83	—	37	93	—	—
1·66	—	34	85	—	—
1·66 repeat	30	—	82	—	120
2·5	—	—	74	—	—
2·5 repeat	—	—	72	—	148
6·64	—	—	52	125	—
6·64 repeat	—	—	—	—	291

In all, 7,000 cells were scored for every mitotic index estimation.

The effect of LSD on the inhibition of cell division is shown in Table 3. At the 74th hour the higher the dose the lower was the mitotic index; however, in cultures stopped at the 53rd hour inhibition was more acute. At the 74th hour, the cells seem to have been overcoming, to some extent, this effect of LSD. Furthermore, when cultures were stopped in the 93rd or 96th hour, the mitotic index was higher than for corresponding controls. Repeat experiments (Table 3) confirmed that the inhibition of cellular division imposed by LSD was gradually overcome.

It is possible that the concentration of LSD decreases as a result of cellular metabolism, and that cells previously inhibited are released from the mitotic block. Cultures with 6·64 μg/ml. of LSD, however, had at the 96th hour a mitotic index three times that of controls

(Table 3), yet the curve for chromosome breakage did not decline for this concentration (Fig. 1).

These experiments do not rule out the possibility that at later stages of culture, LSD or a breakdown product (perhaps present in minute quantities) stimulates cells to divide. It is interesting that rats treated with LSD in early pregnancy produced, in addition to stunted and stillborn young, some unusually large offspring which weighed more than the young of controls ten days after birth[20].

To investigate this fully, peripheral lymphocytes would be inappropriate, for they have only a finite lifetime in culture. A more permanent cell line would be required.

The concentrations of LSD used in these experiments were greater than would be expected in the blood of users of the drug, for it passes quickly into the tissues and is subsequently eliminated. Some organs, however, contain a greater concentration than that originally present in the blood stream[18]. Human cells may well prove to be less sensitive than *Potorous* lymphocytes, but even a far lower level of damage than that observed here could have far reaching results.

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In vitro Cytotoxic Test for detecting Cell Mediated Immunity to Soluble Antigen

THE demonstration that lymphoid cells from immunized donors are cytotoxic for cells carrying antigens to which the cell donor is sensitized has led to the development of *in vitro* tests for cell-mediated immunity[1]. These systems measure directly the lytic activity of "sensitized" lymphocytes, and can be readily adapted to a quantitative approach by incorporating ^{51}Cr (as sodium chromate) into the antigen-carrying (or "target") cell, and following the kinetics of target cell destruction by measuring the release of ^{51}Cr. This approach has been used most effectively in studies of allograft immunity[2,3]. But in spite of many obvious advantages, these cytolytic tests are generally applicable only to systems involving cell surface antigens, and as such have found particular use in the study of histocompatibility systems. I now describe attempts to extend the applicability of these systems to antigens which are not an integral part of the cell membrane but which are covalently linked to it by the use of coupling agents. Similar previous attempts[4,5] have only yielded systems of low sensitivity.

Adult Hartley strain guinea-pigs were immunized, in the rear foot-pads, with 50 µg dinitrophenylated human IgG (DNP–HGG) in complete Freund's adjuvant. Spleens were removed at various times and a viable lymphocyte population was prepared and counted[2]. "Target" cells were prepared by coupling DNP–HGG to mouse mastocytoma cells (P815) of the DBA strain[6] using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide–HCl (ECDI). These cells were especially chosen for their susceptibility to membrane permeability changes and destruction[7]. They have been found, by several criteria, to be more sensitive to lysis than are erythrocytes (ref. 8 and my unpublished observations).

Mastocytoma cells were collected from cultures in modified Eagle's medium supplemented with 10 per cent inactivated calf serum. The cells were washed twice in sterile Tris-buffered saline and 2·0 ml. of the cell suspension at a concentration of 2×10^6 cells/ml. was incubated with 0·2 ml. sodium ^{51}Cr-chromate (specific activity 100 µCi/µg chromium) for 30 min at 37° C. The suspension was then centrifuged at 1,500 r.p.m. for 5 min and washed three times with sterile Tris-buffered saline. Following haemocytometer counting, using a Trypan-blue exclusion test, 10^6 viable cells were suspended in 1 ml. Tris-buffered saline (*p*H 7·2). A solution of DNP–HGG was passed through a 'Millipore' filter (0·21 µm pore size) and 3 ml. of effluent containing 7·5 mg protein in Tris-buffered saline (*p*H 7·2) was mixed with the mastocytoma cell suspension. One minute later, 0·5 ml. of a 500 µg/ml. solution of ECDI was added. Following inversion, the suspension was left at room temperature for 1 h. The cells were sedimented by centrifugation at 1,500 r.p.m. for 5 min and then washed three times with Eagle's medium containing 10 per cent inactivated calf serum. These "coupled" cells were again counted and cell suspensions adjusted to appropriate concentrations.

"Target" cells (10^5 DNP–HGG coated mastocytoma cells) were incubated at 37° C with 5×10^7 lymphocytes from both normal and sensitized animals. The effects of antigen coupling were assessed by measuring ^{51}Cr release from untreated mastocytoma cells and from the same cells following "coating" with DNP–HGG. After incubation for 1, 3, 6 and 9 h at 37° C, 1 ml. of Tris-buffered saline was added to representative tubes which were then spun at 1,500 r.p.m. for 5 min; 1 ml. supernatant fluid was withdrawn and assayed for ^{51}Cr. The result was corrected for the volume left with the cell sediments, which were also counted for ^{51}Cr, and the amount of ^{51}Cr in the supernatant was calculated as a percentage of the total ^{51}Cr which was originally cell-associated. A typical result, using pooled lymphocyte preparations from three normal spleens and from three spleens obtained from animals 8 days after immunization with DNP–HGG, is shown in Fig. 1. Treatment of the mastocytoma cells with DNP–HGG and ECDI did not affect the rate of ^{51}Cr release over a 9 h period. A further study following ^{51}Cr release over a 20 h period similarly revealed no differences between untreated cells and those exposed to antigen and ECDI. The addition of normal lymphocytes caused only a slight increase in ^{51}Cr release (Fig. 1). On the other hand, the addition of lymphocytes from DNP–HGG immunized animals caused a marked increase in ^{51}Cr release from target cells, more than 30 per cent of radioactive label being released during the 9 h incubation period studied, compared with about 12 per cent for "normal" lymphocytes. (A recent adaptation

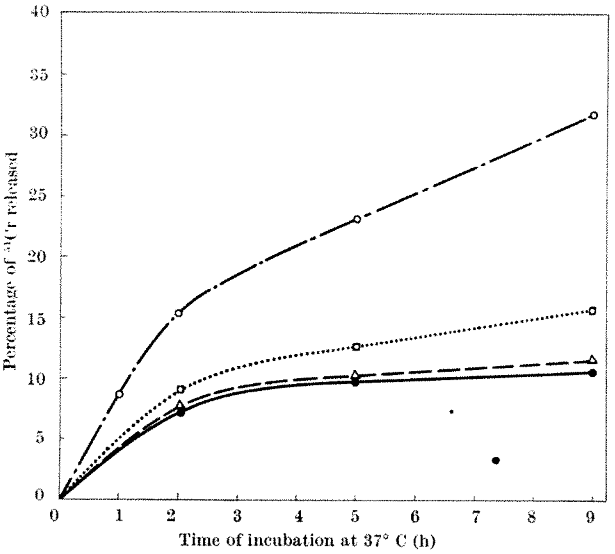

Fig. 1. The rate of ^{51}Cr release from 10^5 mouse mastocytoma cells (●—●), DNP–HGG coupled mouse mastocytoma cells (△ - - △) and from the same number of DNP–Ma cells in the presence of 5×10^7 splenic lymphocytes from unimmunized (□ · · · □) and specifically sensitized (○ - — - ○) guinea-pigs.

in which "target" cells and lymphocytes were gently agitated on a rocking table device has further increased this sensitivity about three-fold.)

The effect of varying the ratio of lymphocytes to target cells in the reaction mixture was investigated by mixing 10^4 target cells with between 10^6 and 3×10^7 lymphocytes from both normal and sensitized animals, in a reaction volume of 0·4 ml. Again, ^{51}Cr release was measured 1, 3, 6 and 9 h after incubation at 37° C. The rate of ^{51}Cr release from target cells was unaffected by the presence of lymphocytes from normal animals (Table 1). There was, however, a direct correlation between the rate of ^{51}Cr release and the number of "sensitized" lymphocytes present: 15·3 per cent was liberated in the presence of 10^6 sensitized lymphocytes (lymphocyte : target cell ratio of 100 : 1), and 33·7 per cent in the presence of 3×10^7 lymphocytes (lymphocyte : target cell ratio 3,000 : 1). The values of lymphocyte : target cell ratios required for extensive lysis in this system are much higher than those observed in systems involving transplantation antigens (compare ref. 7), a finding which almost certainly reflects the low numbers of "sensitized" lymphocytes in the immune spleen following administration of soluble antigen.

Another possible explanation of the rather high ratio of "sensitized" lymphocytes to target cells required for extensive lysis might be that only a relatively small proportion of the mastocytoma cells became "coated" with DNP–HGG in these coupling conditions. Accordingly the antibody induced lysis of DNP–HGG coated mastocytoma cells was studied. To 0·2 ml. (10^5) DNP–HGG coupled mouse mastocytoma cells was added 0·1 ml. of a dilution of guinea-pig antiserum specific for DNP–HGG (approximately 15 μg of antibody). Then 0·1 ml. of a 1 : 2 dilution of fresh rabbit serum was added as a source of complement, and the mixture incubated at 37° C for various times up to 9 h. The rate of ^{51}Cr release was assessed in the usual manner. The cytotoxicity of the antibody and complement preparations was also measured separately (Fig. 2). Lysis by antibody and complement was kinetically very different from that obtained with sensitized lymphocytes (Fig. 1), lysis being complete within 1 h. Lysis induced by either the antibody or complement preparations alone was low. Guinea-pig complement, even in the presence of guinea-pig antibody, was only weakly lytic. These findings (Fig. 2) indicate, however, that at least 80 per cent of the mastocytoma cells have DNP–HGG fixed to their surfaces (this figure can be considered even closer to 100 per cent, for only about 85 per cent of the cell bound ^{51}Cr was released even by distilled water). Thus, unless cytolysis of "target" cells requires a very precise antigenic requirement—for example, a certain number of DNP–HGG molecules on each mastocytoma cell—it seems likely that the lymphocyte : target cell ratios required for cytolysis in this study are high because of relatively low numbers of "sensitized" lymphocytes.

Table 1. EFFECT OF THE CONCENTRATION OF "NORMAL" AND "SENSITIZED" LYMPHOCYTES ON THE RATE OF TARGET CELL DESTRUCTION

No. of lymphocytes in system	No. of DNP–HGG coupled mastocytoma cells (target cells)	Lymphocytes: target cells	^{51}Cr released from target cells after 9 h at 37° C (per cent)
"Sensitized" spleen*			
10^6	10^4	100 : 1	15·3
2×10^6	10^4	200 : 1	22·1
5×10^6	10^4	500 : 1	29·4
10^7	10^4	1,000 : 1	31·8
3×10^7	10^4	3,000 : 1	33·7
"Normal" spleen			
10^6	10^4	100 : 1	12·3
2×10^6	10^4	200 : 1	11·6
5×10^6	10^4	500 : 1	13·2
10^7	10^4	1,000 : 1	12·8
3×10^7	10^4	3,000 : 1	11·4

* A pooled splenic lymphocyte preparation obtained from three animals 10 days after immunization with 50 μg DNP–HGG in complete Freund's adjuvant.

Table 2. SERUM ANTIBODY TITRES AND THE CYTOTOXICITY OF SPLENIC LYMPHOCYTES AT VARIOUS TIMES FOLLOWING IMMUNIZATION OF GUINEA-PIGS WITH 50 μg DNP–HGG IN COMPLETE FREUND'S ADJUVANT

	Cytotoxicity	Serum antibody
Days after sensitization	^{51}Cr released from 10^5 mastocytes by 5×10^7 lymphocytes in 9 h at 37° C (per cent)	DNP–HGG immunoglobulin bound (μg/ml. serum)
0*	12·2	< 20
8	31·8	175
11	29·4	590
24	25·6	1,300
42	22·1	2,800
49	19·6	1,950

* Unsensitized animals (mean of six values).

To assess the cytotoxicity of splenic lymphocytes at various times after immunization with antigen, spleens were taken from groups of animals at intervals following administration of 50 μg DNP–HGG in complete Freund's adjuvant. The amount of ^{51}Cr released from 10^5 target cells by 5×10^7 splenic lymphocytes was assessed following 9 h incubation at 37° C. The capacity of serum samples, obtained at the same time as the spleen excision, to bind

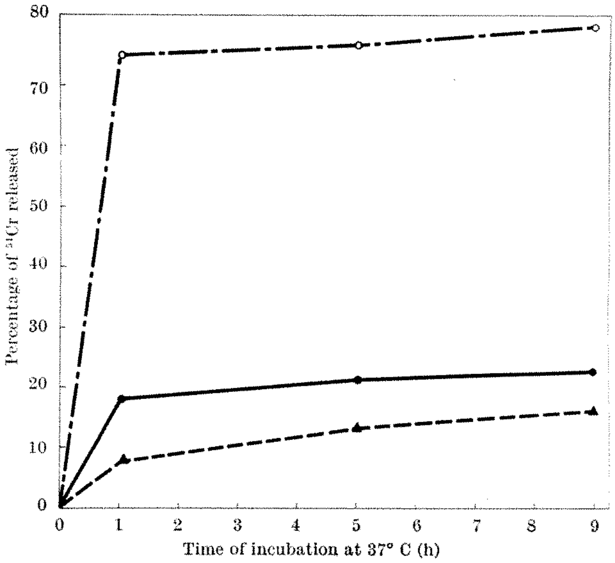

Fig. 2. The rate of ^{51}Cr release from 10^5 DNP–HGG coupled mouse mastocytoma cells by guinea-pig antibody specific for DNP–HGG (▲ - - - ▲) by rabbit complement (●—●) and a mixture of antibody and complement (○ — - — ○).

DNP–HGG was also measured[9] (Table 2). The highest cytotoxic activity was observed between 8 and 11 days after immunization, when serum antibody was low. On the other hand, at a time when serum antibody levels were highest (approximately 6 weeks following sensitization) the cytotoxicity of the splenic lymphocytes, although positive, was rather low. Thus there was no correlation of cytotoxicity with measured serum antibody.

The specificity of the test system was assessed by measuring the cytotoxicity of splenic lymphocytes obtained 8 days after immunization with DNP–HGG for mastocytoma target cells coupled with DNP–HGG, DNP–BSA and HGG. In one experiment, involving pooled lymphocyte preparations from three unimmunized animals and from three spleens obtained 8 days after immunization with 50 μg DNP–HGG in complete Freund's adjuvant, 5×10^7 lymphocytes released 30 per cent ^{51}Cr from 10^5 DNP–HGG coated mastocytoma cells; 22 per cent ^{51}Cr from the same number of DNP–BSA coated cells and 18 per cent ^{51}Cr from the HGG coated cells during a 9 h incubation. The same number of normal lymphocytes released respectively 16, 17 and 15 per cent of the ^{51}Cr from the target cells. When antigens immunochemically unrelated to that used for immunization (for example, BSA) were coupled to the mastocytoma cells, there was no difference between lysis rates in the presence of normal and DNP–HGG sensitized lymphocytes. This indicates that the cytotoxicity has a specificity which is primarily directed against the hapten–carrier complex used for immunization, and as such displays a striking parallel with that of delayed hypersensitivity skin testing in hapten–carrier systems[10].

Further evidence that the cytolytic activity observed here in the presence of "sensitized" lymphocytes was not mediated by serum antibody was the finding that various rabbit antisera specific for guinea-pig immunoglobulins had no inhibitory effect on such lytic activity. The same antisera invariably inhibited lysis mediated by guinea-pig anti-DNP–HGG antibody in the presence of complement. In a typical experiment an antiserum reacting with both the Fab and Fc portions of guinea-pig γ_1 and γ_2-immunoglobulins inhibited >85 per cent the extent of antibody mediated lysis with <2 per cent effect on lysis by "sensitized" lymphocytes.

The modified cytolytic test described here also seems to be specific for cell-mediated immunity, for maximal cytotoxicity was not found to be associated with a maximum serum antibody response. Furthermore, the test was predominantly carrier specific and anti-immunoglobulin antisera failed to cause inhibition of the cytotoxicity.

Because different species may be used to provide target cells and sensitized lymphocytes and as various antigens may be coupled to the target cells, the modified cytolytic test may have general application in the study of cell-mediated immunity.

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Chromatography of Proteins on Dipolar Ion Adsorbants

ROUTINE chromatography of proteins was made possible by the introduction of cellulose ion-exchangers[1]. Protein chromatography on ion-exchangers is technically simple but theoretically complex, there being no quantitative theory to act as a guide for experimentation. Proteins are usually unilaterally distributed between the ion-exchange matrix and the surrounding liquid. They are either quantitatively adsorbed ($K_d = \infty$: $R_F = 0$) or not at all ($K_d = 0$: $R_F = 1$)[2]. Finite distribution coefficients are seldom obtained and only in well specified conditions. Gradient elution[3] is used to make the separation of proteins on ion-exchangers practicable, where the distribution coefficients shift from zero to infinity or vice versa in a concentration interval too narrow to be otherwise adjusted. On the other hand, calculation of the shape and position of the gradient effluent is difficult with present ion-exchange systems, for the gradient is altered continuously during passage through the bed.

We believe that it should be possible to make better adsorbants for protein chromatography; they should contain weak adsorption centres with a relatively high density (100–1,000 μequiv/g) and a hydrophilic matrix. Adsorption could possibly be based on ion-dipole interaction where the dipoles attached to the matrix could be dipolar ions. The electrical field potential χ decreases at a greater rate round dipoles than around unit charges. For an isolated dipole and a single charge the quantities are

$$\chi_{\text{dipole}} = \frac{q \cdot l \cos\theta}{D \cdot r(r + l\cos\theta)}\ ; \quad \chi_{\text{ion}} = \frac{q}{D \cdot r}$$

where q is the positive or negative charge of the dipole or the ion, r is the distance from the positive pole (or from the ion) to the position in the field under observation, l is the distance between the two charges on the dipole, θ the angle between the dipole axis and the line to the point in the field, and D is the dielectric constant of the medium.

One advantage with dipole-ion chromatography is that the internal charge shielding can be closely adjusted by changing the dipole distance l. This can be done, for example, by placing a bridge between the charges. The amphoteric nature of the adsorbant can be expected to introduce interesting consequences in the chromatography of amphotytes as well as of ordinary salts. To evaluate the importance of the charge-shielding effect we carried out experiments using ion-exchangers containing the groups $=\overset{+}{N}H-(CH_2)_n-COO^-$ with $n = 1$, 2 and 5. They were prepared by coupling glycine, β-alanine and ε-aminocaproic acid to 'Sephadex *G*–75' by the cyanogen bromide method[4]. Reversible adsorption of the proteins was found to occur and molecular sieving seems to be more prominent with glycine–'Sephadex', while the weak cation-exchanger effect dominates with the β-alanine and ε-aminocaproic acid derivatives.

As a test substance we used cobra venom (*Naja nigricollis*) from which low molecular weight substances were removed by gel filtration on 'Sephadex *G*–25'. Fig. 1 illustrates a fractionation experiment with 100 mg *Naja nigricollis* toxin protein on a 58 × 1 cm column of β-alanine–'Sephadex *G*–75' (750 μequiv/g) in ammonium acetate buffer (*p*H 6).

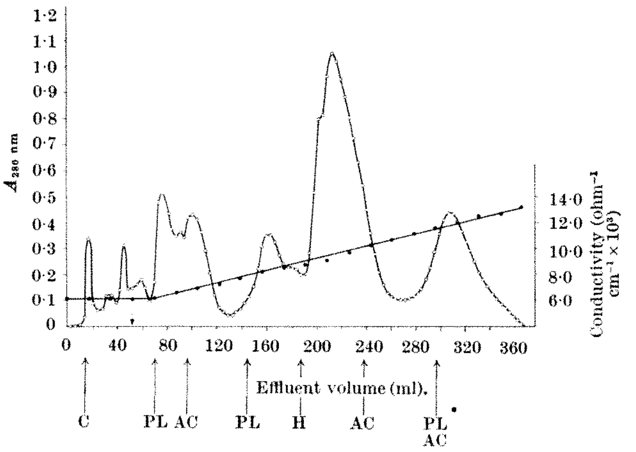

Fig. 1. Chromatography of 100 mg *Naja nigricollis* toxin proteins on β-alanine–'Sephadex *G*–75' (58·5 × 1 cm) in ammonium acetate buffer (*p*H 6·0); starting concentration 0·08 M. Flow rate, 6·8 ml./h. Linear gradient started at arrow. The positions of biological activities are marked below the abscissa: AC, anticoagulant activity; C, coagulant activity; PL, phospholipase; H, hyaluronidase.

The effluent was collected in 3·4 ml. fractions and light adsorption measured spectrophotometrically at 280 nm. The form of the gradient in the eluate was determined conductimetrically. No *p*H changes were observed in the eluate. Some biological activities were determined in or near the peak fractions.

The column referred to in the previous experiment was equilibrated with 0·2 M ammonium acetate buffer (*p*H 6·0) and then eluted with 70 ml. of 0·5 M buffer (*p*H 6·0); there was then a change to 0·12 M buffer (*p*H 6·0). The eluate was collected and the conductivity measured. Equilibration occurs quickly (Fig. 2). Fig. 3 shows a chromatogram of toxin proteins obtained with 0·066 M ammonium carbonate (*p*H 8·0). Most of the proteins move through the column in separated zones and further material is eluted when the buffer is changed to 0·2 M: first there is a displacement peak, and second an elution peak. Representative samples from various parts of the chromatogram were lyophilized and analysed by polyacrylamide electrophoresis. More strongly adsorbed components generally migrated more rapidly to the cathode, although striking deviations occur. Amino-acid analysis was carried out on some of the peak fractions. The arginine content has a dominating influence on the peak position, but a low arginine content can be compensated for by a high lysine content. The marked differences in the distribution of material between the two chromatograms reflects a striking variation in the influence of different structural factors in both the gel and the sample substances.

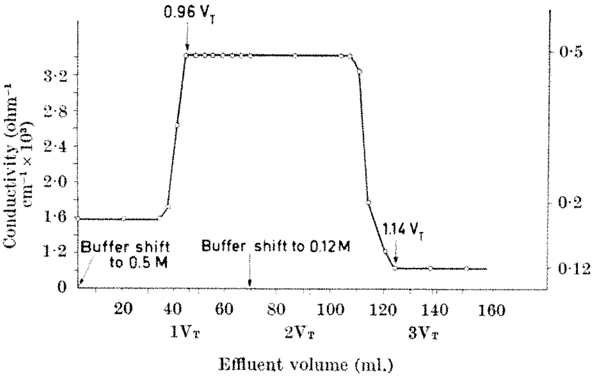

Fig. 2. Equilibration with buffer on a column (58·5 × 1 cm) of β-alanine–'Sephadex G–75'. Ammonium acetate buffer (*p*H 6·0); total bed volume 46·2 cm³.

The dipolar adsorbants have the following useful chromatographic qualities. First, a relative insensitivity to variation in *p*H but a great sensitivity towards ionic strength (which can easily be controlled). Good separation can be obtained using shallow buffer concentration gradients at constant *p*H. Second, suitable conditions—at least for low molecular weight basic proteins—can readily be found for linear chromatography with good reproducibility. Third, high capacity with moderate ionic strengths (with ammonium acetate, for example, ionic strength 0·1) in the *p*H range 4–9. Fourth, in all cases tested proteins can be reversibly adsorbed with moderate changes in buffer concentration or *p*H. The risk of denaturation during chromatography is therefore slight or non-existent. Fifth, equilibration is rapidly attained after buffer changes; the concentration gradient moves through the bed with little delay (Fig. 1). This is of practical importance and may even hold promise for technical development. Sixth, dipolar ion adsorbant beds have a lower tendency to alter in volume on change to another medium than is the case for ordinary ion-exchangers.

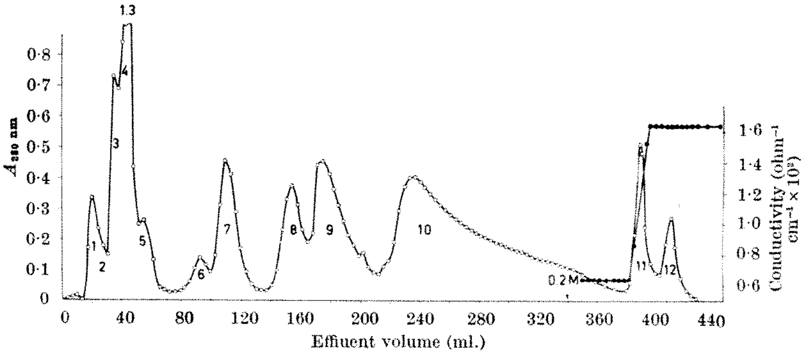

Fig. 3. Chromatography of 100 mg toxin proteins on a column (58·5 × 1 cm) of β-alanine–'Sephadex *G*–75' at *p*H 8·0 in 0·066 M ammonium carbonate buffer.

Improved separation of gram quantities of crude venom may be achieved by chromatography on a larger scale. Possibilities for adjusting the chromatographic operation interval by introducing other types of charged groups, such as guanidino and sulphonate, are being investigated at present. It remains to be seen to what degree dipolar adsorbants can complement or replace the conventional ion-exchangers.

We thank the Swedish Board of Technical Development for financial support and Mr Conny Eklund for assistance. Patents are pending on the technique.

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Esterase Activity of Chymotrypsin on O-DNP-L-Tyrosine Ethyl Ester Substrates

WHILE developing a chemical method for the assay of ACTH by dinitrophenylation and subsequent hydrolysis with chymotrypsin, we observed that no di-DNP-seryl-tyrosine could be identified in the digestion mixture by gel filtration on 'Sephadex G-25' column and thin layer chromatography. This suggested that DNP-ACTH is attacked by the enzyme, not at the tyrosyl-2 peptide bond, but at other positions yielding large peptide fragments. The O-DNP derivative of tyrosine may interfere with the action of chymotrypsin on the tyrosyl peptide bond. No work seems to have been reported on the esterase activity of chymotrypsin when the phenolic group of tyrosine is blocked by alkylation or acylation. To verify our hypothesis, model substrates were synthesized in which the phenolic group of tyrosine was blocked by a DNP residue.

N-Acetyl-O-DNP-L-tyrosine ethyl ester was synthesized from N-acetyl-L-tyrosine ethyl ester by Sanger's reagent. A colourless crystalline solid was obtained after recrystallization from aqueous ethanol and had the following characteristics: m.p. 113°–114° C; infrared absorbance peaks, in cm^{-1} (KBr): 1,730, 1,740 (carbonyl); 1,650, 1,600 (amide); 1,560, 1,350, 850 (nitro); found C, 54·46; H, 4·44; N, 9·88—$C_{19}H_{19}O_8N_3$ requires C, 54·67; H, 4·58; N, 10·06 per cent. No hydrolysis of this substrate with chymotrypsin could be detected by a potentiometric method, even after incubation for 12 h, while the corresponding free phenolic compound is the usual substrate for chymotrypsin with a K_m value of $6\cdot9 \times 10^{-4}$ M (ref. 1).

Because this substrate was sparingly soluble in aqueous medium, further evidence was sought by synthesizing a soluble substrate, N-maleyl-O-DNP-L-tyrosine ethyl ester. N-maleyl-L-tyrosine ethyl ester was synthesized by maleylation of L-tyrosine ethyl ester using the procedure of Sheehan *et al.*[2]. An oily product was obtained which could not be crystallized, but which was purified by high voltage electrophoresis in a buffer of pyridine–acetic acid–water (25:1:225, *p*H 6·5), at a voltage of 2 kV for 1·5 h. The pure maleyl derivative was eluted from the paper with acetone yielding an oily material. Its purity was checked by high voltage electrophoresis and thin layer chromatography. This material was directly dinitrophenylated in the usual way giving a colourless solid: m.p. 58°–60° (decomp.); infrared absorbance, in cm^{-1}, (KBr) 1,720, 1,740 (carbonyl); 1,650, 1,600 (amide); 1,560, 1,345, 850 (nitro); found C, 53·03; H, 4·40; N, 8·43—$C_{21}H_{19}O_{10}N_3$ requires C, 53·28; H, 4·02; N, 8·87 per cent. N-Maleyl-L-tyrosine ethyl ester was easily cleaved by chymotrypsin (K_m, $2\cdot08 \times 10^{-2}$M), but the corresponding O-DNP derivative resisted hydrolysis.

These experiments strongly suggest that, for chymotryptic cleavage at the tyrosyl bond, the phenolic group must be free. It would be interesting to test model substrates in which the DNP group was replaced by groups having a negative inductive effect with varying degrees of hindrance.

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Marijuana and Immediate Memory

CLINICAL anecdotes and recent studies suggest that a marijuana "high" interferes with immediate memory (ref. 1, and unpublished manuscripts by L. Clark and A. Wikler). We describe here the effects of calibrated amounts of tetrahydrocannabinol (THC) on cognitive tasks which require immediate memory.

We used THC that had been extracted from marijuana supplied by the Bureau of Narcotics and Dangerous Drugs. Thin layer and gas liquid chromatography of these extracts showed predominantly 1-Δ^1-THC, with some cannabidiol and cannabinol. Quantitative calibrations were made so that doses (20, 40 and 60 mg) were based on the actual content of 1-Δ^1-THC. The doses were in the range which, for synthetic 1-Δ^1-THC administered orally, invariably produces clinical effects approaching those of psychotomimetic drugs, such as lysergide[2,3]. A placebo was composed from an extract of marijuana with cannabinoids removed.

The subjects, who were fasting, received their doses in a randomized order unknown either to them or to the testers. Four oral doses, in 95 per cent ethanol diluted with water, were given on four separate experimental days at least one week apart. The eight subjects were college-educated males in their twenties, weighing between 63 and 85 kg, who had been screened for good physical and mental health. All had smoked marijuana, but at no greater frequency than once a month. They were tested before the drug was given, and at 1·5, 3·5 and 5·5 h afterwards. All testing was done individually in an austere research laboratory.

The digit span task required subjects to repeat, either forwards or backwards, a series of random digits read by the experimenter at a rate of one a second. The span was the longest number of digits that could be reproduced without error on two successive test trials. If the subject's attention waned, the investigator restarted the trial to determine accurately the upper limit of the subject's capacity for that test period.

Table 1. DIGIT SPAN AND THC DOSES TESTED BY ANALYSIS OF VARIANCE (d.f. = 3,21).

THC dose	Means Digits forward	 Digits backward
Placebo	9·0	7·8
20 mg	8·2*	6·9*
40 mg	8·1*	6·6*
60 mg	7·9*	6·4*
F	4·5	5·2
Significant	$P < 0\cdot05$	$P < 0\cdot01$

* These values significantly differ from placebo at $P < 0\cdot01$ by *t*-test for correlative means, 2-tail.

Table 1 shows that significant differences between placebo and each of the THC doses were obtained for both forward and backward digit spans. There were, however, no significant differences between effects of 20, 40 and 60 mg doses, indicating that the smaller dose of THC impairs immediate memory and that larger doses do not significantly augment the impairment. The most significant decrement in forward memory occurred 1·5 h after drug ingestion, with a return almost to the pre-administration result at 3·5 h. For backward memory, there was a greater mean decrement over doses and the impairment was still present 3·5 h after ingestion.

We also gave three subjects a more complicated digit reordering test in two stages. We first read a list of random digits, shorter by two numbers than the subject's forward memory span before he had taken the drug that day, and asked him to recite the digits in the same order. We then instructed the subject to arrange the digits in serial rank order, either upwards or downwards. Each of the three subjects was given at least two reordering tasks during the four testing periods of each experimental day. In the non-drug state (with placebo and before taking the drug), subjects made nineteen errors in forty trials. In the drug state (1·5 and 3·5 h after ingestion for all doses), they made forty-eight errors in thirty-five trials. This difference between the total number of drug and non-drug errors was significant (Mann–Whitney Test).

In the non-drug state, all nineteen errors involved the omission of a given digit or the insertion of one not given. In the drug state, there were thirty-seven errors of this type. In the drug state only, there were also eleven errors in which the subject did not carry out instructions given a few seconds before: for example, he would reorder from low to high rather than follow the instructions to reorder from high to low.

Our observations, like those of others[4], indicate that impairments in immediate memory from marijuana intoxication do not follow a smooth time-function but, rather, are episodic, brief in duration and not always under volitional control. These episodes often interrupted the speech patterns of our subjects and seemed to be associated with the intrusion of extraneous perceptions and thoughts (compare ref. 5). One subject commented: "Well, I just felt very confident and able to remember the numbers . . . then when it came time to give them back, I'd see and hear, mostly see, I think, all kinds of numbers. And I wouldn't know which ones were the right ones." Another subject commented: "I can understand what I'm thinking, but I can't understand what I'm saying. . . . Because there are so many thoughts in between what I'm saying . . .".

The cognitive impairments induced by THC also involve intermittent lapses in attention. One subject stated: "Everything's pretty mixed up . . . I lose track of what's been happening. And it sometimes even seems like a long time has passed, but I know it hasn't, because I'm generally still in the middle of a word or sentence". Because we exhorted our subjects to try as hard as possible, we feel that they were able considerably to mitigate the influence of impaired attention on their cognitive performance. Usually the cognitive changes abated in 3 to 5 h, although some subjects described residual impairment up to 24 h later.

Similar intermittent defects in remembering relevant events may contribute to various types of behavioural disorders, including acute psychoses[6,7]. Elsewhere, we discuss correlations between THC-induced impairments in immediate memory and other behavioural changes[8].

This work was supported by grants from the US Public Health Service (to L. E. H.), the National Institutes of Mental Health (to F. T. M.), and Stanford University (to J. R. T.). We thank Dr Helena Kraemer, Miss Cathy Owen and Mr Saul Kantor for their assistance.

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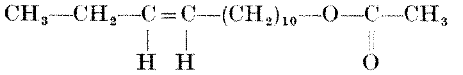
Sex Pheromone of the Oblique-banded Leaf Roller Moth

ALTHOUGH at least ten pheromone compounds have been reported for lepidopterous species, only three[1-3] have proved to be true sex attractants in natural field conditions. We now report that the structure of the sex attractant of the oblique-banded leaf roller *Choristoneura rosaceana* (Lepidoptera, Tortricidae), one of the most widely distributed North American tortricids, is *cis*-11-tetradecenyl acetate (I).



$$\mathrm{CH_3{-}CH_2{-}\underset{\underset{H}{|}}{C}{=}\underset{\underset{H}{|}}{C}{-}(CH_2)_{10}{-}O{-}\underset{\underset{O}{\|}}{C}{-}CH_3}$$

(I)

Two years ago, after identifying the sex attractant of the red-banded leaf roller moth (*Argyrotaenia velutinana*) as (I), we trapped many oblique-banded leaf roller males along with red-banded leaf roller males in orchard traps baited with this compound. We established a laboratory rearing programme for oblique-banded leaf rollers and processed 20,000 female abdominal tips as before[2,3]. At least a hundred-fold increase in free attractant was obtained by saponification of the crude extract followed by acetylation. Identification of the active component was accomplished by the usual techniques[2,3] of column, gas and thin-layer chromatography, ozonolysis and mass spectrometry.

Synthetic compound (I) was very stimulatory to males in the laboratory and has attracted hundreds of oblique-banded leaf roller males in field trapping experiments in the past two years. The *trans* isomer also elicited a response in laboratory bioassays until it was fractionated twice on silver nitrate-impregnated silica gel thin-layer plates to remove traces of the *cis* isomer (I), as was the case for the false codling moth[4]. The *trans* isomer had no inhibitory or masking effects in the laboratory bioassay, but was found to be a very potent inhibitor to male response in the field when added in low percentages to the attractant (I). This emphasizes the importance of field bioassays for biological activities which are not evident in the laboratory.

Because both oblique-banded and red-banded leaf roller males are attracted to the same compound in the field, it is not clear how specificity is effected. The two species have overlapping seasonal and diurnal cycles and share the same host plants. The addition of dodecyl acetate to (I) does effect partial reproductive isolation by drastically increasing attractancy for red-banded leaf roller males[5] and decreasing it for oblique-banded leaf roller males. Preliminary studies indicate that other compounds can give the opposite effect when added to (I) in enhancing oblique-banded leaf roller attractancy and inhibiting red-banded leaf roller response. The emission of different secondary compounds by females of each species along with the sex attractant (I) could effect reproductive isolation in the field for these two species.

We thank F. Wadhams, C. D'Augustine and M. Holland for rearing, sexing and snipping thousands of female moths for this project. This research was supported by a US Public Health Service research grant from the National Communicable Disease Center, Atlanta, Georgia.

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Book Reviews

A THEORY OF HUMAN KNOWLEDGE

Genetic Epistemology

By Jean Piaget. Translated by Eleanor Duckworth. (Woodbridge Lectures, Columbia University, 1968.) Pp. 84. (Columbia University Press: New York and London, April 1970.) $5; 45*s*.

In his autobiography Professor Piaget has related how, after his first book on children's reasoning had been published, Einstein asked him to undertake "a developmental study to determine whether the child's intuition of speed depended on a previous comprehension of time and space or whether the former came first"; he found (as he puts it) that "the child is a little relativist, not a Newtonian absolutist". The fruitful suggestion thus thrown out by Einstein turned his attention to what he now calls a "genetic theory of knowledge, i.e. the project of a scientific epistemology based on mental development, both ontogenetic and phylogenetic"; and the problems that emerged formed the starting point for numerous experimental studies carried out by himself and his co-workers at Geneva during the past forty years.

The detailed results have already been set forth in three volumes entitled *Introduction à l'Épistémologie Génétique* (1950); and the four Woodbridge Lectures, now issued in an English translation, present a brief summary of his chief conclusions, and report several new and ingenious experiments confirming his original contentions. The first lecture is chiefly critical. Present-day philosophers, he maintains, tend to treat scientific knowledge as "something final and static"; they either ignore the psychological basis of all human knowledge or improvise a psychology of their own regardless of the actual findings of psychological research. The remaining lectures deal with the formation of such concepts as substance, class, number, time and space. Generally speaking, their gradual development in the mind of the child runs parallel to their gradual evolution during the history of scientific thought. But there are notable exceptions; thus the average child of seven can recognize the topological properties of space, but he does not appreciate Euclidean or projective properties until three years later.

For this reason, so Piaget believes, a merely historical approach may be highly misleading. Why, it is often asked, is *Homo sapiens* distinguished from all other animals by the ability to reason? Because, so it is commonly said, he is endowed with the power of speech. The logical positivists, for example, hold that "all mathematical concepts are derived from logical concepts, and that logical concepts are essentially linguistic structures". Quite wrong, replies Piaget. Observations on both normal children and deaf-mutes reveal that "many logical concepts develop much earlier than, and independently of, language; the idea of number also develops, more or less independently, out of practical attempts to arrange things in visible order". This, as he points out, is in line with Chomsky's contention that "language is derived from reason, not reason from language". But he doubts Chomsky's further assumption that "logical concepts and the ability to reason are innate": "they develop progressively during the first few years of life out of the preverbal coordination of actions; their processes are gradually internalized and so give rise to such conceptual structures as order, number, class and the like".

Piaget, however, explains that he is here concerned solely with the ontogenetic, not with the phylogenetic, development of human knowledge, and therefore refrains from going farther back into comparative biology; if he did so, he might (so it has been argued) find some justification for Chomsky's view. We may certainly agree with Piaget that, so far as individual development is concerned, human knowledge is dependent on, and limited by, the sensory and motor endowment of the human organism. But that endowment must in turn have been developed by natural selection during the evolution of the race.

A human being is a body of moderate size moving about with moderate speeds on dry land, where the objects with which he has chiefly to cope are likewise solid or semisolid bodies of a similarly moderate size, moving (if at all) with similar speeds. In such an environment survival is most effectively secured by a species endowed with vision as the chief distance-sense and kinaesthesis (the muscle-and-joint sense commonly confused with touch) as the sense that chiefly guides his movements. Man therefore tends to interpret the universe in terms of the motion of material bodies moving about in a visible space. Until quite recently this was the source of the basic concepts, not only of common sense, but also of physical science. The revolutionary changes in physics, quite apart from the philosopher's criticisms, have brought home to us how limited and inadequate these concepts really are. As Piaget himself expressed it in one of his earlier papers, "Man's hereditary nervous system and sense-organs are essentially the product of his primitive adaptive needs".

Cyril Burt

LONDON'S COUNTRY

London's Green Belt

By David Thomas. Pp. 248. (Faber: London, March 1970.) 65*s*.

The 846 heavily protected square miles of London's green belt are one of the most remarkable fruits of town and country planning in Britain during the past 25 years. The existence and purpose of the belt seem to be widely known and understood, and politicians believe that they dare not allow a brick to be layed upon its fair green face. A measure of their nervousness is the three years it took to get 400 acres of derelict glasshouses in the Lea Valley allocated to house building between 1963 and 1966. Only the protection of open countryside is pursued with comparable zeal by Britain's planners.

The effect of this care, as Dr David Thomas says in his well researched book, is that the spread of residential and commercial land uses grew by only 0·3 per cent between 1955 and 1960. At that rate, open land in the belt will drop from its present 88 per cent to 82 per cent during the next hundred years. For a stretch of real estate under pressure for development from one of the greatest cities in the world, that is extraordinary.

One gets a sight of what all this means by taking the Bakerloo underground line to Stanmore. There, as you step out of the train, is London *circa* 1938. Barely a thing has changed since. You can walk out of the station, cross a main road, go down a close of quasi-Bauhaus houses, climb over a farm gate and you are in fields. By any standards you are also in an ideal building site.

Dr Thomas describes how the green belt was first proposed at the turn of this century as a pleasure ground for

Londoners or a circular parkway. But it was in the 1930s when Middlesex was disappearing at unprecedented speed beneath bricks and mortar that ideas gave way to action. A sense of urgency was added by the publication of figures about the shortage of playing fields within London and the rate at which potential parkland was being developed on the fringes of the city. These gave strength to Raymond Unwin's 1933 proposal for a thin green girdle which found its political destiny in the London County Council's first Labour administration in 1934. Money to buy open fields and then a special Act of Parliament to protect them appeared during the next four years.

The green belt of today stems from the more extensive one proposed by Abercrombie in 1944. It was given statutory backing between 1954 and 1958, but no sooner had this happened than the Home Counties, ever fearful of London's urban hordes, proposed extending it to cover 2,000 square miles.

Dr Thomas sets out to consider how London might have grown without a green belt but says that there is insufficient evidence on which to theorize. He contents himself with a statistical description of land use changes in the belt and then another one of all the land, urban and rural, in a 10 mile ring around built-up London.

These analyses throw interesting light on things such as the uneven distribution of recreation land, nearly twice as much to the south-west as to the south-east, and on the great extent of the towns set in the green belt to the west of London. Questions about the future of the belt, whether it has impeded London's dynamic, whether it should be turned into a chain of new Royal Parks dotted about with weekend cottages for Londoners, are hinted at but not explored. Dr Thomas leaves the answering of them to the policy makers, which is a pity, but he has provided a useful base from which to start.

TERENCE BENDIXSON

PRESSURES ON THE COUNTRYSIDE

People and the Countryside

By H. E. Bracey. Pp. xx + 310 + 8 plates. (Routledge and Kegan Paul: London, March 1970.) 63*s*.

DR BRACEY has written a comprehensive survey of man's influence on landscape, ranging from the earliest trackways along the chalk uplands to the modern nature trail, from the struggles of a small population to clear forests for agriculture to the demands of a huge population for recreation. Careful scientific studies of the problem of the invasion of the countryside are comparatively recent. How many tramping feet will destroy the marram grass on sand dunes ? What measures can be taken to reduce erosion ? What steps can be taken to lessen the impact of too many people watching too little wild life ? Bracey gives well documented accounts of research on these, now burning, questions.

Bracey looks at every aspect of the countryside, workwise as well as playwise, the repercussions of new industry, railways, bus services, local government and, of course, the effect of new roads and traffic. The internal combustion engine is bringing greater changes to the landscape and the countryside than anything which has happened in all the centuries which Bracey surveys. The straightening and widening of roads, the building of by-passes and car parks, are profoundly affecting our land, and it is essential that some overall policy should be adopted, essential that at some stage road engineers should stop, at least in places such as the National Parks and Areas of Outstanding Natural Beauty, if the character of these is not to be completely changed. Bracey says rather less on this subject, and on the possibilities of traffic management (such as the Goyt Valley scheme), than might have been expected.

There are chapters on dereliction and litter; on the problems of water supply, including the use of reservoirs for recreation; on common land, its present and future use; on the increasing role of forests in providing facilities for recreation, and there is comment on the large part the Forestry Commission has played in pioneering this use. Every chapter is followed by comprehensive notes giving the sources of information. There is, in addition, as an appendix, a list of further reading.

This is a book, as the author himself says, for the thoughtful citizen who sees changes coming rapidly to the countryside and wishes to know what is being, and what can be, done about it. It is also a book for the student and teacher, providing a firm and adequate basis for further research. Bracey's practice of enumerating his points, which he does throughout the book, makes for an easy assessment of the problems, which can then be pursued in more detail. It is a book also for all those concerned with the countryside, planner, forester, water engineer, those in local government and those concerned with leisure. It is certainly a book which should be on many bookshelves; it will, after the initial perusal, be frequently required for reference.

GEOFFREY BERRY

HISTORY OF BRITISH PLANTS

Studies in the Vegetational History of the British Isles

Edited by D. Walker and R. G. West. (Essays in Honour of Harry Godwin.) Pp. 266. (Cambridge University Press: London, April 1970.) 160*s*; $25.

THIS is a book of eleven essays written by thirteen people, all of whom were at some time associated with the subdepartment of Quaternary research in the botany school at the University of Cambridge. The collection is published to mark the retirement from the chair of botany at Cambridge of Professor Harry Godwin, who founded the sub-department and was for many years its inspiration and director. Most of the contributions directly reflect Professor Godwin's own special interests, although it may truly be claimed that it would be difficult to find an aspect of Quaternary research which had not been at some time the subject of original contributions by Professor Godwin himself.

Mention should perhaps first be made of the essay by Professor D. Walker on estimates of rate of change in autogenic hydroseres and of the use by Professor F. Oldfield of evidence from stratigraphical and pollen analyses to investigate the ecological relationships of vegetation at Blelham Bog, because both these contributions recall Professor Godwin's original work in plant ecology and particularly in the ecology of bog and fen. Similarly, Dr J. J. Donner's essay on changes in the relative levels of land and sea in Scotland recalls Professor Godwin's papers on the same problems around the coasts of England and Wales.

The book contains such a wide variety of interests that a short review must unavoidably be superficial or selective. The first essay, by R. G. West, provides a very concise but valuable summary of the history of British vegetation throughout the whole Pleistocene period. It emphasizes the enormous quantity of information that is now available and yet the comparative lack of knowledge of regional variation in all phases earlier than the Late Weichselian and Flandrian, as we must now call the Late and Postglacial periods.

Mrs T. G. Tutin's contribution is also a masterly review and summary of a large amount of information from the English Lake District from which there are now available pollen-diagrams from sixty sites. It must be hoped this will inspire others to write regional summaries. Dr A. G. Smith discusses evidence that Mesolithic man had a greater influence on vegetation than is generally recognized, and Miss J. Turner examines the use of pollen analysis of several profiles in one site to locate local forest clearance and separate it from regional patterns.

Two essays deal specifically with Professor Godwin's interest in the history of the British flora. The essay by Professors G. F. Mitchell and W. A. Watts brings together and discusses knowledge of the history of the Ericaceae in Ireland and of the rarer Irish species in western Europe. Miss A. P. Conolly and Dr E. Dahl present a detailed analysis of the distributions of arctic-alpine and montane species of flowering plants in Britain in relation to the estimated mean maximum temperatures on the summits of the highest hills. This is an extension of Dahl's earlier work in Scandinavia. Where a particular species occurs, the correlations are often remarkable, but many species fail to appear in large areas where summer temperatures seem to be suitable. Why is *Arctostaphylos* not in Wales or on the moors of Devon and Cornwall ? Is it not a little worrying that this plant should favour the warmest south-westerly exposures in most of its English localities and grows in profusion on sunny slopes in the Cevennes associated with *Buxus sempervirens* and *Helleborus foetidus* ? Dare one suggest that if summits more than 3,000 feet above sea-level or corries above 2,500 feet were plotted on a map they might also be correlated with particular limits of mean maximum summer temperature ? One is left with an urgent desire to do some experiments with the plants themselves. C. D. PIGOTT

PLANT CHEMISTRY

Organic Chemistry of Secondary Plant Metabolism

By T. A. Geissman and D. H. G. Crout. Pp. ix + 592. (Freeman, Cooper: San Francisco, 1969.) n.p.

A NUMBER of books similar to this have appeared during the past 5 to 6 years, and although there is nothing excitingly original in this new volume it can be fully recommended. It is well planned, attractively presented, covers a wide field and is relatively free from errors. It represents a solid achievement which will be of great value to the increasing number of organic chemists taking an interest in problems of biosynthesis. It will also appeal to biochemists, not only because of the information provided but also because the biochemical difficulties inherent in biosynthesis are more fully appreciated than they are in some other texts. Indeed, in chapter two the authors give an outline of the biochemical activities which provide the primary building stones for the formation of the complex natural products they discuss. This chapter should be read in conjunction with a biochemical text.

The authors have, on the whole, achieved their claim that "the general principles of chemistry of natural products" have been described "upon which students may build a more detailed and extensive familiarity with one or more of the individual classes that make up the compounds of Nature". From this point of view, the chapters on flavonoids, oxidative coupling of phenols and alkaloids are particularly successful. The chapters on terpenoids are satisfactory as far as they go, but they seem to be less up to date than many of the other chapters. It was unfortunate that in a book whose preface is dated April 1969 it was not possible to mention the discovery of squalene 1,2-oxide as the precursor of triterpenes, or to discuss the basic stereochemical investigations on sterol biosynthesis initiated by Conforth and Popják.

One of the pleasures associated with this book is that almost every time one opens it some statement or comment sets one off on a new line of thought. I, for one, am grateful to the authors for taking the time and trouble to set out in this book their considerable knowledge of and insight into this fascinating field of chemistry. T. W. GOODWIN

TERMITE SOCIETY

Termites

A Study in Social Behaviour. By P. E. Howse. Pp. 150 + 4 plates. (Hutchinson University Library.) (Hutchinson: London, March 1970.) 35*s* boards; 15*s* paper.

THE behaviour of social insects is perhaps too often described in terms of the life of the honeybee. The appearance of an introductory book on termite biology is therefore welcome; as the author says in his preface, many undergraduates know termites better as the home of symbiotic protozoa than as insects. There are disadvantages, however, in approaching sociality in insects through the termites (or for that matter through ants or wasps). The varied life histories of different species compound the complexity which already exists in the life of a single species, and this becomes more serious still when the patchiness of research makes it necessary for discussion to skip from one species to another. Termites are even more tropical in distribution than other social insects and, as a result, have attracted less investigation, except perhaps of an economic kind. Lastly, the developmental stages obtrude more into accounts of social biology in termites than in Hymenoptera; probably most readers will have to turn backwards or forwards to the tables of developmental stages rather frequently.

Dr Howse has been quite successful in meeting these problems. After two slightly repetitive chapters on the habits of termites and elements of termite society, he deals with three aspects of termite life: the swarming and mating of sexual forms, communication by sound and scent and the form, and to a smaller extent the functions and construction of nests. Clearly in some other areas our knowledge is very small; termites are, after all, subterranean and cryptic animals. A more detailed account of tandem formation and of food exchange should, however, have been possible.

The final chapter discusses the relation of some general views of animal behaviour to the social behaviour of insects. This too is successful as far as it goes, but the fact is that our knowledge of the behaviour of termites is at present inadequate for the production of concepts, at least at the "physiological" level which Dr Howse (and I) favour.

The book is adequately but not copiously illustrated, though some illustrations have been marred by over-reduction in size. There are some 170 references and an index. This book can be recommended as a welcome source of information for undergraduates, and others, who are interested in behaviour or entomology.

J. H. SUDD

FISH IDENTIFICATION

Diagnostic des Pièces Rachidiennes des Téléostéens et des Chondrichthyons

1 Gadides. By Georges Desse and Marie-Henriette du Buit. Pp. 71. (Le Expansion Scientifique: Paris, 1970.) n.p.

IDENTIFYING isolated fish vertebrae is a difficult task, even when good collections of comparative material are available. Thus, one should welcome the publication of this new series whose aim is to provide diagnostic characters for identifying vertebrae to the species level.

The first volume is a short, largely pictorial account of various vertebral characters in fourteen species of cod-like fishes from north European seas. The authors have worked out a system of identification based on ossification patterns in the centra, visible externally or in transverse section. The patterns are illustrated by photographs and somewhat schematized line drawings. There are also radiographs (mostly in frontal section) of each element in the entire vertebral column. These are intended to supplement the photographs and drawings (based on one abdominal and one caudal centrum) which form the bases for the diagnostic vignettes.

I have tested out the identification scheme proposed in the book against identified skeletons and with material from an Anglo-Saxon midden. The results were varied, and showed up several flaws, particularly in presentation. For example, no scale is given for any figure (except the radiographs); many of the photographs are so lacking in contrast as to be useless, and the corresponding drawing is often too simplified to be critical. Where the drawings are naturalistic, no account seems to have been taken of individual or ontogenetic variation; indeed, nowhere is there any record of the number of individuals on which the diagnoses were based. Lack of contrast, the small size, and poor quality reproduction greatly reduce the value of the radiographs which, in fact, could be the most useful of all the figures.

In spite of these criticisms, I believe that the system developed by Desse and du Buit could be of great value, especially if in future publications the quality of the photographic reproduction is improved, or the photographs be replaced by good half-tone drawings. For the moment, the worker faced with the need to identify centra will still rely heavily on comparative specimens.

P. H. GREENWOOD

MARINE BIOLOGY

Marine Biology

An Introduction to its Problems and Results. By Hermann Friedrich. Translated from the German by Gwynne Vevers. (Biology Series.) Pp. xii + 474. (Sidgwick and Jackson: London, January 1970.) 70*s* boards; 25*s* paper.

To the modern student of marine biology the sea is presented as a dynamic system, emphasis being given to the interactions between the atmosphere, the oceans and the continental margins which influence the vast production cycles on which its economy is based. This demands a constant awareness of the physical and chemical conditions within the environment, in so far as they affect the biological systems in which the student is interested. This information must be gathered from a wide variety of sources, many written outside a biological context, and in drawing these together in a book, Dr Friedrich has performed a valuable service.

Principally an ecologist, the author gives a short historical introduction dealing with the great expeditions, collecting gear and the major ecological divisions of the sea. This is followed by widely ranging chapters on the adaptations of the plant and animal communities of the pelagic and benthic environments to the physical conditions they experience.

Although he claims the book is written for the non-specialist, it is obviously aimed at the advanced student of marine biology, and the twenty pages of references emphasize the thoroughness with which the author has documented his material. It is particularly useful as a guide to the European literature which might otherwise be neglected through language difficulties.

It is perhaps inevitable that in attempting to cover so wide a field the author's responsibility for independent critical appraisal is sometimes overlooked and the text becomes a mere compendium. For example, Tucker's theories on eel migration are acknowledged but not described, and (p. 166) a remarkable suggestion that the deep-water fish *Opisthoproctus* drifts upside down with its tubular eyes gazing into the depths is accepted without question. My students found the section on temperature/salinity relationships of water masses in relation to animal distribution so brief as to be incomprehensible without consulting the original authors.

Dr Vevers has provided a clear and fluent translation, and the text is well illustrated with 206 figures and 56 tables (including one which has to be inverted to read it). In spite of these shortcomings, the value of the book lies in the presentation of material previously available only as original papers, and at this price the paperback edition represents a real bargain.

DEREK DORSETT

VERTEBRATE BRAINS

Neurobiology of Cerebellar Evolution and Development

Edited by R. Llinás. (Proceedings of the First International Symposium of the Institute for Biomedical Research: AMA Education Research Foundation.) Pp. x + 931. (American Medical Association: Chicago, 1969.) n.p.

THIS large, well produced book is a record of a symposium held only a little more than a year ago. With so much material, some attempt to organize the contributions into a logical pattern was clearly desirable, and this has been carried out partly by grouping the contributions according to phylogeny—one section dealing with fish, one with amphibia and reptiles, and another with mammals. A separate section is devoted to ontogeny, and two papers are included under the heading "Vestibulo-acoustic Input".

The book opens with an introduction, of laudable intention, comprising chapters on vertebrate history (Romer), comparative cerebellar morphology (Elizabeth Crosby) and on the comparative anatomy and physiology of the cerebellar cortex (Llinás and Hillman), and ends with a review by Jansen. The general standard of contribution is high, and one would have no reservations about recommending the volume as a collection of individual papers. Does it not, however, purport to be something more ?

That the fashionable word "neurobiology" appears in both title and preface suggests this, and the inclusion of the chapters by Romer, Crosby and Jansen confirms it. There is, also, the opening of the introductory chapter by Llinás and Hillman: "Since this symposium is dedicated to the study of phylogenetic development of the cerebellum . . ." (p. 43). Such may have been the intention but, inhibited perhaps by the warnings of both Romer and Crosby on the difficulties of extrapolating "lines" of evolution from the data of comparative studies, the participants have in fact produced a series of excellent papers on various aspects of the comparative structure and function of the cerebellum. The contributions that do attempt some generalization or synthesis in the direction of this "dedication" do so from limited viewpoints. Thus Llinás and Hillman conclude that the evolution of the cerebellar cortex becomes readily comprehensible if one contrasts the development of the climbing and mossy fibre systems in relation to the control of Purkinje cells. No attempt is made to correlate such changes with other changes in the organization of the cerebellar system, or alterations in the locomotion of evolving animals. One might object that too little is known to enable us to do this, but that itself is worth demonstrating in this context.

This deficiency is significant enough to highlight the compartmental nature of the text, and brings into question the reason for publishing the proceedings of symposia in this form. From a perusal of the titles and declared intentions of many group meetings of this general kind, one might be forgiven for supposing the point of publishing the contributions as collections to be an attempt to distribute to a wider audience some measure of a synthesis or "state of knowledge" of the topic. Even if one assumes that the participants were able to reach such an enviable state of assimilation, this is rarely, if ever, communicated by the published volume. The present book is no exception, but it must again be stressed that this is no reflexion on the papers read as individual contributions.

K. E. WEBSTER

DRUGS AND SYNAPSES

The Pharmacology of Synapses

By J. W. Phillis. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 43.) Pp. xi + 358. (Pergamon Press: London and New York, 1970.) 120*s*; $16.

Our understanding of signalling processes across synaptic junctions in the nervous system has shown exciting progress in the past decade and it is discoveries in this period which the book describes. The author provides a factual and comprehensive catalogue of what is now known of transmission at a wide range of synapses, but the order in which the material is presented is not ideal, with initial chapters on important general characteristics of possible transmitters at all sites. This is followed by chapters on transmission in the peripheral and central nervous systems of mammals and, following this, there is an account of invertebrate and molluscan synapses. This arrangement requires the reader to make frequent back references to obtain relevant metabolic and morphological information.

As a review this book is a notable success and the bibliography is excellent. Where the book fails and frustrates is in not providing interpretations or possible explanations of the frequently controversial information. There are numerous examples of this, for instance, when describing presynaptic transmitter actions, apparently contradictory experiments are quoted without comment or an attempt to equate the anomalies. This lack of critical approach has detracted particularly from the author's assessment of the status of possible transmitters at various sites. The criteria which should now be satisfied by a substance before it can be classed as a transmitter have been listed but have been couched in ambiguous terms and the author does not state which, if any, are essential. This indecision has allowed the author to make strong assertions as to the identity of the transmitters at many sites, including the lateral geniculate nucleus, mitral cells and cerebral cortex, although many important criteria for each example have yet to be demonstrated. As the author himself points out, there are good reasons why extreme caution must be exercised in the identification of a neurotransmitter.

Many of the chapters lay great emphasis on the iontophoretic application of drugs to single neurones and, although some of the difficulties inherent in this technique are mentioned, the unpredictable quantities of many substances ejected is not remarked on. This may explain some of the divergent results which the author has attributed to anaesthetic effects or even to fluctuations in environmental conditions. Other important techniques have often been dealt with in a cursory fashion. Notable gaps occur in the uncritical description of *in vitro* studies and in describing the variety of information available on the evoked release of potential neurotransmitters.

Although the book disappoints in its unbalanced reporting and unimaginative interpretation, there is no doubt that it forms a valuable source of information and provides a most comprehensive and much needed review of a complex and important subject.

J. F. Mitchell

PEROXIDE REACTIONS

The Determination of Organic Peroxides

By R. M. Johnson and I. W. Siddiqi. (Monographs in Organic Functional Group Analysis, Vol. 4.) Pp. ix + 119. (Pergamon Press: Oxford, London and New York, 1970.) 50*s*; $6.75.

Aerobic oxygen reacts with most types of organic compound to give organic peroxides in low concentration. Their structures and properties are almost as diverse as those of the organic monoxy compounds, and a wide variety of methods have been used in their analysis. This book serves as a guide to the most suitable procedure.

After an introductory chapter on the occurrence of organic peroxides, the various types are classified and their reactivities are related to their structures. This is oversimplified: there are some ten different mechanisms by which peroxides can react, and a compound which is unreactive by one mechanism may be very reactive by another.

The various types of chemical and physical analytical methods (for example, iodometric, colorimetric, polarographic and chromatographic) are then discussed. Detailed procedures are quoted for the recommended methods, and examples of their applications are tabulated together with data such as half-wave potentials, R_f values, and retention times. This is a useful, thorough, and critical compilation, and the best source of reference which is available in this field.

There are some shortcomings in nomenclature. Names such as succinic acid peroxide, phthalyl peroxide, and diisopropyl hydroperoxide are not very informative, even if they were used by the original authors. A number of half-truths also occur, which should not be taken at their face value. For example (p. 9), it is stated that "peresters are formed when alcohols react with peracids"; this may be possible in principle, but such a reaction has never been established.

These are minor points, however, and do little to detract from the value of a very useful review.

A. G. Davies

DOWN'S SYNDROME

Measurement of the Facies

A Study in Down's Syndrome. By Michael Joseph and Carol Dawbarn. (SIMP Research Monograph, No. 3.) Pp. xii + 114. (Spastics International Medical Publications in association with Heinemann (Medical): London, 1970.) 50*s*; $7.50.

Dr J. Langdon H. Down, in a paper published in the *London Hospital Reports* for 1866 and entitled "Observations on the Ethnic Classification of Idiots", wrote that a very large number of congenital idiots are typical Mongols; so marked is this that, when placed side by side, it is difficult to believe that the specimens compared are not children of the same parents. Down further wrote that the face is flat and broad, and destitute of prominence; the cheeks are rounded and extended laterally; the eyes are obliquely placed and the internal canthi more than normally distant from one another; the palpebral fissure is narrow, the nose is small.

This classic description of the Mongol child was based on careful clinical observations, but clinical impressions may be inaccurate or misleading and require confirmation by methods involving exact measurements. The research monograph by Joseph and Dawbarn describes an original approach based on painstaking measurements of the face, eyes, nose, mouth and ears, and will prove invaluable to paediatricians and others who may be called upon to diagnose Down's syndrome; furthermore, the techniques can be used to define more accurately other disorders associated with an abnormal facial appearance.

The authors review the attempts from Aristotle (383 to 322 BC) through Albertus Magnus (AD 1192 to 1280), Leonardo da Vinci (1452 to 1519) and Peter Camper (1722 to 1789) to Topinard (1830 to 1911) to measure the human face. These anthropologists and others were interested chiefly in bony landmarks, but the methods devised by Joseph and Dawbarn also take account of the distribution of the soft tissue of the face.

Twenty infants within six months of birth in whom the diagnosis of Down's syndrome had been confirmed cytogenetically were studied in detail, together with twenty normal "control" infants attending an infant welfare clinic. Each infant was photographed in the true antero-posterior and in the lateral position using a 35 mm single lens reflex camera. Each infant was then given a sedative and an impression of the face was made using alginite, alum and plaster bandage, and from this impression a mask was cast. Landmarks agreed by anthropologists were used as points of reference and measured with callipers and the contours were measured with strips of dental wax. For comparative purposes the detailed measurements of facial structure were adjusted to a common age of 190 days. Detailed measurements obtained either from the photographs or from the face mask were made of the general contours of the face, eyes, nose, mouth, ears and facial profile, and the results obtained were compared with those recorded in the literature from 1866 by Langdon Down to Gustavson in 1964.

A few examples will suffice to reveal the exactitude and attention to detail recorded in this monograph. The eye was assessed by the length, angle, asymmetry and shape of the palpebral fissure, by eye size and prominence, by distance between the eyes and by the epicanthic folds. The ear was assessed by length, shape, prominence, lobe and pattern. These and other detailed measurements of facial structures will prove of great value in diagnosis, for they supersede a purely qualitative description and they will be helpful in defining the cardinal features of Down's syndrome and other disorders which have a specific facies. The investigation shows that within their own group the Down's syndrome children are subject to as wide a range of individual variation as normal children.

The authors are to be congratulated on the original, careful and painstaking way in which they have obtained accurate measurements of facial characteristics; their work forms a springboard for extensions of observations by subsequent workers.

R. McL. Todd

ORGANIC REAGENTS

Reagents for Organic Synthesis

Vol. 2. By Mary Fieser and Louis Fieser. Pp. 538. (Wiley (Interscience): New York and London, March 1970.) 160*s*.

The second volume of the Fiesers' book is as welcome as the first. As a result, no doubt, of much correspondence and much searching, many new entries are made dating both from before the deadline of the first volume (1966) and after it. The first volume has lived up to the usefulness I claimed for it in my review (*Nature*, **216**, 1253; 1967); now the second volume containing, as it does, much extra information about reagents already in the first volume will obviously be just as useful. Almost every reagent I set out to look for I found (the exceptions were trichloroethanol and cupric fluoroborate). I look forward to further volumes in what I hope is a series. These books are useful personal possessions as well as essential for a working library.

Ian Fleming

REVISED DURRANT

Introduction to Advanced Inorganic Chemistry

By P. J. Durrant and B. Durrant. Second edition. Pp. xv+282. (Longmans: London, February 1970.) 160*s*.

This is a new and enlarged edition of a book that appeared in 1962. Inorganic chemistry has not remained still in the nineteen-sixties and this book has changed with the times to include plenty of new and up to date material. The authors are husband and wife, a chemist and a physicist, both greatly interested in education and teaching. They have used tables, diagrams, symbols and other devices of presentation which will make the book valuable for students.

The early part is devoted principally to presenting the hypotheses of valency and chemical bonding and the experimental procedures that provide information about atomic and molecular structure. This section includes an interesting chapter on group theory which was not present in the earlier edition. The more theoretical part of the book is much more lengthy and complete than might have been anticipated, but it forms a useful basis for the rest of the book. In the more factual chapters later on in the book, the diagrams, for instance of structures, are good, and considerable use has been made of tables to present a lot of information compactly and to demonstrate similarities and differences.

Since I have had this book in my office and home I have used it for obtaining information several times and it has given good service. I have also found, on these occasions and as I have gone through it, that it is interesting because it derives not only from the knowledge of the authors but also from their attitude and thinking; and this gives the book character.

J. W. Linnett

PRINCIPLES AND ADVANCES

Principles of Radiation Chemistry

By J. H. O'Donnell and D. F. Sangster. Pp. viii+176. (Arnold: London, March 1970.) 45*s*.

Advances in Radiation Chemistry

Vol. 1. Edited by Milton Burton and John L. Magee. Pp. xi+401. (Wiley (Interscience): New York and London, March 1970.) 155*s*.

When a new branch of chemistry can boast a book entitled "Introduction to" and now followed by "Principles of", then I suppose it has achieved a measure of respectability. The authors of *Principles of Radiation Chemistry* argue in the preface that radiation chemistry is now far from being a narrow specialization, and that the study of the effects of radiation on chemical systems provides information about the mechanisms and rates of chemical reactions, particularly those which involve free radicals and ions, which are not readily investigated by conventional methods. The subject is therefore suitable and is increasingly being introduced into undergraduate and graduate courses, to which this book is primarily directed. A difficulty, however, is that the subject has not developed systematically and for many of the diverse systems investigated—organic, inorganic, either in the solid, liquid or gaseous state—often the only unifying factor is that each has been subjected to the influence of ionizing radiation. The chemical effects and mechanisms are often as diverse as is the chemistry of such systems.

Bearing this fundamental difficulty in mind, the authors have succeeded in producing a valuable and concise outline of the entire subject. Following a most extensive survey of the literature sources, the basic modes of interaction of radiation with matter are outlined. There follows a survey of the experimental procedures and special techniques used although, clearly, the fast reaction methods have applications in other areas of chemistry also. The chemical effects are divided into those found in gaseous systems, aqueous solutions, organic compounds and polymerization and graft copolymerization. There is a chapter also on radiation damage in relation to biological systems. Allied studies such as hot atom chemistry and the self-decomposition of labelled molecules are also discussed, and there is a general chapter on the developments such as radiation sterilization and industrial applications which have grown up alongside radiation chemistry.

Yes, the authors have succeeded in a very difficult task, and radiation chemistry generally may well benefit more from this elementary text outlining simply the basic principles than from the many symposia and advanced specialized treatises which have recently appeared.

Advances in Radiation Chemistry is a new series which will be devoted to "developments in the various specialized areas fundamental to theory and experiment in radiation chemistry". This publication follows some 2 years after *Radiation Reviews*, which covers a somewhat similar field, although the latter publication's terms of reference are rather broader than "radiation chemistry". In practice, both publications have published reviews on identical topics already, although only four contributions are included in this first volume of *Advances*. Indeed, one author, Dr J. K. Thomas, has written about the related subjects, solvated electrons and the radiolysis of water, for both review publications. Moreover, one member of the advisory board of *Advances* is himself an editor of yet another review series *Current Topics in Radiation Research*, which also allows reviews in radiation chemistry, although during its development has tended to concentrate on radiation developments more allied to biology.

It would be unrealistic, therefore, not to pose the question whether this subject, barely 30 years old as a discipline, can sustain such a barrage of reviews. Only time, of course, will truly answer this question, but my own view is that provided subjects are critically reviewed and that the frequency of publication is controlled by the developments in any one area, no conflict need arise. This latter point, I note, is also stressed by the editors of *Advances* in their introduction to the series, and as pioneers in the field, they are in an excellent position to view developments with perspective. Even the most obvious duplication, as has occurred with Dr J. K. Thomas's contributions, need present no waste of effort. For example, the author has in the first contribution dealt with a limited aspect of the subject of more current interest, and now given a comprehensive panoramic view of the radiation chemistry of water, which is incidentally of the highest quality. In their own way both reviews can serve a useful function.

Other subjects reviewed in *Advances* are "Charged Particle Tracks and their Structure", by A. Mozumder; "Theory of Transient Negative Ions of Simple Molecules", by Joseph C. Y. Chen and "Chemical Synthesis by Ionizing Radiations", by C. W. Wagner. The first volume, therefore, is disproportionally weighted towards theory, but no doubt future volumes will seek to correct the balance.

Ignoring, therefore, the groans of the librarians and the strains on departmental funds, it is possible to welcome the new venture and wish it well.

GLYN O. PHILLIPS

KINETICS OF CHANGE

Mass Transfer in Heterogenous Catalysis

By Charles N. Satterfield. Pp. xvi + 267. (MIT Press: Cambridge, Massachusetts, and London, February 1970.) 133*s*.

IN presenting a complete revision and extension of his earlier book[1], Professor Satterfield has managed to repeat his tradition of writing a standard text in the field of catalysis. For all those interested in different aspects of the kinetics of catalytic reactions, the book must be required reading.

The general approach is, first, to present the concepts qualitatively, and then to build a quantitative picture on this basis, treating both the simple case and the more complex situations. Theoretical treatments are given, but emphasis remains on their quantitative application to various practical problems of reaction engineering. Several illustrative examples are used throughout the text, and many available experimental data are summarized in tables and graphs.

Molecular diffusion, diffusion in pores and the estimation of diffusion coefficients are considered in the first chapter, together with a brief description of the properties and methods of characterization of solid catalysts. Mass transfer in fixed beds, fluid beds, and various gas-solid-liquid systems is reviewed, and the concept and measurement of effectiveness factors are described. The arguments are applied to simple systems and to more complex cases, particular attention being paid to the influence of temperature gradients in a pellet and to problems introduced by complex kinetics. The final chapter is concerned with the poisoning and regeneration of catalysts and with a short section on the influence of diffusion on selectivity. This book is longer than the earlier volume, the section on mass transfer with chemical reaction having particularly increased in length. It was somewhat disappointing that the discussion of selectivity is still limited to a short section, rather than being treated as a basic concept, and that no discussion of the merits of stirred tank reactors is presented, but these are minor faults.

The book is expensive, but it should interest final year undergraduates, research workers and design and development engineers.

D. L. TRIMM

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[1] Satterfield, C. N., and Sherwood, T. K., *The Role of Diffusion in Catalysis* (Addison Wesley, New York, 1963).

BASIC OPTICS

Handbook of Gas Laser Experiments

By G. L. Rogers. Pp. 67. (Iliffe: London, March 1970.) 30*s*.

THIS little book is a most timely and useful work which is recommended to all concerned with introducing students to the fascinating world of modern optics. Moreover, those who have entered this field from the disciplines of microwave engineering will especially welcome the pieces of experimental "know-how" which are not part of their training.

It consists of a concise collection of some eleven basic experiments in coherent wave optics.

Brief but sufficient background (with references) is given as an introduction to each experiment and some specimen results are also given. As expected, simple experiments in holography are included; in particular, it is also pleasing to see experiments on the zone plate, Fourier images and Fraunhofer diffraction by single and repeated apertures, thus providing examples of wave phenomena spanning microwave antennas to X-ray crystallography.

ALAN P. ANDERSON

FRINGE PATTERNS

Multiple-Beam Interference Microscopy of Metals

By S. Tolansky. Pp. ix + 147. (Academic Press: New York and London, March 1970.) 45*s*.

THIS book is concerned with principles, experimental techniques and applications of multiple beam reflexion fringes of equal thickness. There are thirteen chapters, together with a collection of references to the work of the author's school, a brief appendix on fringes of equal chromatic order and a useful index.

The principles are treated largely in a descriptive way with a minimum of theory, but there is much useful detail about experimental techniques. The chief value of this book, however, is undoubtedly in the wealth of examples of the use of the beautiful technique of multiple beam surface topography. Professor Tolansky can justly claim to be the sole originator of this simple and elegant method, and in this book we can see some of the great variety of applications which he has pioneered in the past three decades. The following are some of the lesser known applications: electropolished single crystals, loaded ball-bearings, growth spirals on silicon carbide, surface roughness caused by etching on cleaved surfaces, micro-cleavage effects, pile-up round various surface hardness testing indentations, and machining surface quality. The title of the penultimate chapter, "Barton's Button", refers to a historical curiosity, some early 19th century metal buttons which were cast with a coarse grating ruling pattern as a novelty for the dandies of the day; interferometry was used to determine the depth and profile of the rulings. Plastic moulded gratings have recently become available as ornaments on cufflinks, brooches, earrings, and as "skin jewels" for attaching to the forehead, arm cheek, and the like. *Plus ca çhange . . .*

The book is beautifully produced, as one expects from the publisher, and the half-tones of fringe patterns are superb. It can be recommended as an excellent addition to the bookshelf of anybody with an interest in interferometry.
W. T. WELFORD

ATLAS OF THE HEAVENS

Smithsonian Astrophysical Observatory Star Atlas of Reference Stars and Nonstellar Objects

Prepared by the staff of SAO. Pp. ix + 13 + 152 star charts. (MIT Press: Cambridge, Massachusetts, and London, March 1970.) 173*s*.

CONSIDERING the wide range of uses for which charts are needed in optical astronomy, the number available is not excessive. The two most widely used classical atlases are the *Bonner Durchmusterung* or *BD* catalogue and charts (with their southern visual and photographic extensions by the Cordoba and Cape Observatories respectively) and the Palomar Observatory—National Geographic Society Sky Survey, which has no general catalogue and is so rich that it is unlikely ever to acquire one. Astrographic catalogues and charts cover large zones of the sky to 14th magnitude (photographic) north of declination −25°, but have coordinates inconveniently expressed in x and y and no data on galaxies and nebulae, while the Franklin-Adams charts (with no catalogue) provide a poor man's sky survey that is still useful south of the Palomar survey's declination limit at −33°. Other more widely available charts exist (for example, Norton's *Star Atlas* and the Skalnate Pleso atlases by A. Bečvar) and often provide a great deal of useful information, but they are limited either to fairly bright stars or to restricted zones of the sky.

Recent developments in astronomy and space research have increased the demand for rapid access to stars of accurately known position for optical tracking of solar-system objects and space vehicles, for the preparation of finding charts and for the identification of radio, X-ray and infrared sources on the Palomar charts or on photographs taken with large telescopes. The *BD*—an extraordinary achievement in its time and still very useful after 100 years—is virtually complete to the 9th magnitude and has many fainter stars as well, but it has many disadvantages. Its coordinates refer to the epoch of 1855 and often are inaccurate by one or two arc minutes—in some cases even more. Its format is also unsuitable for use at the telescope. At large observatories, the tendency nowadays is to prepare appropriate charts or overlays for an interesting region by computer, using standard catalogues in machine-readable form. But, naturally, these facilities are not always available, and even when they are they may not be usable at short notice, for example, when one is suddenly asked to observe an Apollo vehicle that is getting into difficulties. Consequently the production of a good modern set of charts covering the whole sky, like the present *SAO Star Atlas*, is a very welcome development.

This atlas is designed to be used in conjunction with the *SAO Star Catalog* (1966), which gives accurate positions (epoch 1950·0) and proper motions for some 250,000 stars and is almost complete to the 9th magnitude. (Some fainter stars are also included, but much fewer than in the *BD*.) Thus every star in the atlas (apart from about 1,000 additional stars of unknown proper motion in the South Polar Cap) has a precise position available and so is automatically suitable as a reference star for natural and man-made objects in the solar system or for the preparation of overlays for the Palomar charts and other photographs. In addition, galaxies (with the correct size and orientation), globular clusters, planetary nebulae and NGC objects of other categories are shown, denoted by special symbols. The scale is 0·863 cm per degree, matching that of photographs taken with the Baker–Nunn cameras, and transparent interpolation réseaux enable positions to be read off directly to an accuracy of about one arc minute. Prepared from the *Catalog* with the aid of an analogue computer, the *Atlas* is divided into 152 11 inch × 14 inch charts on stiff paper which can easily be handled at the telescope. This convenience is paid for to some extent by the rather small scale, which makes it advisable to use a magnifying glass, especially in a poorly lighted dome.

At just under 1*s* 2*d* per chart, this excellent atlas is very reasonably priced and it can be most highly recommended to amateur and professional astronomers alike.
BERNARD PAGEL

DECISION MAKING

Decision Theory

By D. J. White. (The Centre for Business Research, University of Manchester.) Pp. ix + 185. (Allen and Unwin: London, November 1969.) 48*s*.

THIS book is essentially a report of the findings of a research project carried out by the author while at the Manchester Business School. The project was concerned with the content of decision theory and its importance in practical decision making. The chief emphasis is placed on an examination of the various concepts which have been introduced in studying the problem of decision. It must be said that the whole subject is only at an interim stage in its development, and there seem to be very few firm conclusions.

A long chapter is devoted to theories of choice, value and uncertainty. Then, after a chapter on decidability, there are some practical considerations in decision analysis. These include a comparison between the physical and human sciences, and it is at this point particularly that one realizes that the subject matter of decision theory consists of a subtle complicated area of human thought which is, at the same time, the concern of the physical scientist, the behavioural scientist, the logician and the philosopher. After a chapter on pragmatic aspects of decision theory, the book concludes with a chapter on mathematical models and decision.

It must be said that the author has written what, to many people, will appear as a learned account of a difficult subject. If anybody hopes that his personal powers of decision making might be improved by reading the book, he is likely to be disappointed. There is no gentle introduction to the subject matter; and, indeed, the first sentence in the text occupies no less than seven lines of print. The reader, if he is to understand the account of value, will have to be able to think of value as an order preserving homomorphism, and to know what is meant by a boolean algebra of sets. Also, in order to understand the discussion of uncertainty the reader will need some knowledge and appreciation of the Heisenberg principle of uncertainty, as expounded in the theory of quantum mechanics. All this is very exciting, but it is only for real scholars, with considerable background knowledge in diverse fields, and with real mental span. It is in the nature of the subject, and the author is to be congratulated on the masterly way in which he has bridged the void between the physical and human aspects of the subject.
L. S. GODDARD

Correspondence

Reprint Request Reflexes

SIR,—I cannot entirely agree with Drs Davies, McKenzie and Turner (*Nature*, May 30) that our spoof letters produced remarkably few reprint requests. My letter to you printed on May 30 was written on April 8 and the number of requests has now reached 111, while a few more come in each week. Requests for the original "middle article", the genuine one, came to 986. If one assumes that this number included about 111 from people who had not read the article (probably an underestimate), then the ratio of "phoney" to "genuine" requests is at least 1 to 8. This ratio varies considerably from country to country; the worst (highest) is from Germany at 16/36, and Czechoslovakia (9/28) where it may be difficult to get the journal. Other continental and Scandinavian countries are much better (14/148) and USA is slightly worse than average (60/434). As one would expect, the ratio for UK and Commonwealth countries is low (8/174), but from these countries came the largest number of jocular cards such as the one from a Dr Sidney Arbour-Bridge . . . not included in the reckoning.

Yours faithfully,

V. R. PICKLES

University College,
Cardiff.

Deservingly Franked

SIR,—I was interested to read the results of recent experiments on reprint request reflexes by Dr Pickles and by Dr Davies and his collaborators (*Nature*, May 30).

My own policy is to send reprints in the first place to friends and colleagues known to be working in the same field and to people requesting them by letter; to people in places I would like to visit; and, of course, to those whose cards bear a pretty stamp.

Yours faithfully,

W. J. MARSHALL

University College Hospital Medical School,
University Street,
London WC1.

Not a Killjoy

SIR,—As the brutes who "pulled up by the roots" the "delicate flower" planted by Drs Davies, McKenzie, and Turner (*Nature*, **226**, 881; 1970), we too regret the apparent failure of their plan to count and identify the corps of foolish reprint-requesters. Objectively, of course, they and Dr Pickles might have been right in ascribing this negative result to *Dateline in Science's* "gratuitous republication" of their original announcements of this intention, so we tried to visualize just how our 125,000 readers—only physicians in active practice, and all of them in the United States—could manage to "do [their] cause no good".

Somehow, the image was blurred: we couldn't imagine very many American physicians reading *Dateline*, suddenly recognizing the impending *faux pas*, and quickly instructing the nurse scanning *Current Contents* not to request that piece on prostaglandins. The population enacting this scenario seems rather limited.

Indeed, Sir, it occurs to us that, if the test failed, it was not because of our "withering blast of publicity" but because of the experimental "design". Assuming one thoughtless pest dispatched a postcard, it may be said that the test succeeded—but would Davies *et al.* and Pickles seriously contend that they could anticipate an optimum or maximum number of such respondents? How then can they claim that *Dateline's* report inhibited any significant portion of an indefinable group? Why then are they disappointed?

Perhaps Turner and his fellow applied mathematicians at Cambridge will devise a workable programme that can really flush out malingerers who acquire reprints to avoid paying 4*s* weekly; should they do so, we pledge to keep their experiment secret.

Yours faithfully,

NAT HALEBSKY

Dateline in Science, Inc.,
575 Madison Avenue,
New York, NY 10022, USA.

Spin-off from Cancer Research

SIR,—I would like to comment on your quotation (*Nature*, **225**, 991; 1970) from *Science is God* by David Horrobin.

Probably it is true that cancer will not be understood until more is known about fundamental processes going on inside the cell. But since the time of Claude Bernard the idea that toxic compounds are useful tools in the study of fundamental processes has often been proved true. To take only one well known example, toxic antibiotics have been widely used to investigate the mechanism of synthesis of macromolecules. Was it ever suggested that actinomycin or puromycin should not be studied until after the synthesis of nucleic acids and protein had been elucidated? Similarly with compounds causing cancer. It was work with carcinogens which led to the discovery of DNA repair enzymes in mammalian cells, and thus revealed the occurrence of unsuspected fundamental biochemical processes. Incidentally, the ingenuity of the experiments is evidence for the scientific ability as well as for the dedication of the scientists concerned.

To mention only one other aspect of the subject, the rate of progress in the study of biochemical events controlling differentiation has been very slow. If cancer is a disease of differentiation, study of how carcinogens cause a change in cell type may help to throw light on this process.

Yours faithfully,

VALDA M. CRADDOCK

MRC Toxicology Unit,
Medical Research Council Laboratories,
Woodmansterne Road,
Carshalton, Surrey.

Gold on the Moon

SIR,—Your Astronomy Correspondent states (*Nature*, **226**, 580; 1970) that the glazing within small lunar craters is an "awkward" discovery. Not so. It is generally our understanding of a new phenomenon which is awkward, or in the wrong direction.

Accepting the cogency of most of Professor T. Gold's explanation, but not the solar flare, I should like to suggest that the source of the radiation was the Earth's ionosphere during the last geomagnetic reversal (700,000 years BP). The energy to heat the ionosphere was derived from the ions accelerated by the new magnetic field increasing from zero. The total energy requirement to glaze the Moon rocks on this hypothesis is less than 10^{-5} that required by the solar flare hypothesis. Two predictions can be made to support this hypothesis: (1) the age of the glazing on the rocks is 700,000 years; (2) the intensity of the glazing effect is diminished with distance from the sub-Earth (sub-Tellurian ?) point on the Moon.

Yours faithfully,

C. W. KAYSING

2244 Sunset Crest Drive,
Los Angeles, California 90046.

Solubility of Life

SIR,—My attention was arrested by a sentence (*Nature*, April 11, 1970) in the paper "Biological Molecules in their Excited States" by Eisinger, Lamola, Longworth and Gratzer, all presumably physicists: "The biologically interesting conditions are, of course, room temperature and aqueous solution. . . ."

A marine biologist might object to "room temperature". But is it possible that physiologists have been treating cells as solutions for so long that physicists are taking it for granted that this is the state of living matter ?

Yours faithfully,

H. R. CATCHPOLE

Department of Pathology,
University of Illinois.

This letter has been shown to the authors of the paper concerned, one of whom replies as follows: *Mea culpa, mea minima culpa!* We physicists should never take sides in arguments between biologists. We were unaware that an issue existed, but we hope the physiologists will not scorn to pursue the hare that Professor Catchpole has started.

Announcements

University News

Four new professorial appointments have been made at **Brunel University: Dr G. C. Bond,** Johnson Matthey and Company, has been appointed to the chair of applied chemistry; **Dr P. Feltham** has been appointed to a new chair of applied physics; **Dr P. Macdonald** has been appointed to the chair of statistics and becomes head of the new Department of Statistics and Operational Research; **Dr T. F. Slater,** University College Hospital Medical School, has been appointed to the chair of biochemistry.

Professor John T. Dunlop, chairman of the University Committee on Governance and a member of the Committee of Fifteen, has been appointed **dean of the Faculty of Arts and Sciences in Harvard University.**

Two members of the faculty of the **Massachusetts Institute of Technology** have been appointed Institute professors. **Professor Arthur T. Ippen** is head of the Water Resources Division of the Department of Civil Engineering and director of the Water Resources and Hydrodynamics Laboratory; **Professor Franco Modigliani** is professor of finance in the Sloan School of Management and professor of economics.

The personal title of professor of tribology has been conferred on **Dr William Hirst,** senior lecturer in the Department of Applied Physical Sciences and head of the Unit on Lubrication and Wear in the **University of Reading.**

Dr J. K. A. Beverley, reader in medical microbiology, has been appointed to a personal chair in the Department of Medical Microbiology, **University of Sheffield.**

The Cancer Research Centre of the **University of Tehran School of Medicine** has been extended to include a hospital unit, to deal with surgery, radiology, haematology and chemotherapy. **Professor A. Habibi** has been appointed director of the new centre, which has been named the **Tadj Pahlavi Cancer Institute.**

Appointments

Mr H. R. Galleymore, manager of the Newcastle Technical Centre, Proctor and Gamble Ltd, has been appointed a member of the **University Grants Committee.**

Dr P. C. Young, head of the Chemical Department of the British Standards Institution, has been appointed first secretary-general of the **Comité Européen de Coordination des Normes.**

The Board of Governors of the **International Atomic Energy Agency** has appointed **Dr Kurt Waldheim,** former Minister for Foreign Affairs of Austria, as chairman of the Safeguards Committee, and **Mr J. A. K. Qartey** (Ghana) and **Academician Brunó F. Straub** (Hungary) as vice-chairmen.

Miscellaneous

Awards from the **Lady Tata Memorial Fund** for research in leukaemia have been made to **T. M. D. Dexter,** Manchester; **D. J. Reen,** Dublin (full-time scholarships); **T. J. E. Rytomaa,** Helsinki; and **B. Szafarz,** Paris (expenses grants); the awards held by **J. F. Dore,** Paris, **Y. Najean,** Paris (expenses grants); **O. Mach,** Prague, and **P. Stryckmans,** Brussels (part-time scholarships) have been renewed for the academic year 1970–71.

In view of the present lack of knowledge of the distribution of seaweed—an important factor in marine pollution—the **British Phycological Society** will collaborate with the **Biological Records Centre** in mapping the seaweed distribution around the British Isles. The first stage will be the collation of record cards which will be sent in by collectors. Those wishing to participate in the scheme as collectors should apply to Dr T. A. Norton, Department of Botany, The University, Glasgow W2.

CORRIGENDUM. In the article "Netherlands: Stop and Go on the Nuclear Merry-go-round" (*Nature*, **226**, 1020; 1970), the figure given in the right-hand column of p. 1021, line 22, should be Dfl. 550,000,000, not Dfl. 550,000.

ERRATUM. An amendment should be made to the erratum (*Nature*, **226**, 782; 1970) referring to the article "Increase in Antiviral Activity of Polynucleotides by Thermal Activation" by E. de Clercq, R. D. Wells and T. C. Merigan (*Nature*, **226**, 364; 1970). The sentence beginning on line 16 of the paragraph following Table 1 should read "Addition of 10^{-3} M EDTA following the 37° C incubation step did reverse the activating effect whereas rapid cooling to 0° C did not".

International Meetings

June 28–July 2, **Health Physics Society Annual Meeting,** Chicago (Professional Associates, 6710 Clayton Road, St Louis, Missouri 63117, USA).

June 29–30, **Optimization Techniques in Circuit and Control Applications,** London (The Manager, Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2R OBL).

June 29–July 10, **The Molecular Basis of Biological Specificity and Control Mechanisms,** Seattle (Battelle Seattle Research Center, 400 NE 41st, Seattle, Washington 98105, USA).

July 3, **Polymer Composites,** Cardiff (Organizer of Short Courses, University of Wales Institute of Science and Technology, 22 North Road, Cardiff CF1 3DY).

July 23–24, **Materials Forming Processes,** Swansea (Dr J. P. Berry, Rubber and Plastics Research Association, Shawbury, Shrewsbury, Shropshire).

August 31–September 3, **Fluid Mechanics and Design of Turbomachinery,** University Park, Pennsylvania (Department of Public Information, Pennsylvania State University, 312 Old Main Building, University Park, Pennsylvania 16802, USA).

September 8–10, **Optical Techniques for Investigations in the Upper Atmosphere,** Exeter (Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Furnished 3 bedroom house in or near Redhill, Surrey, required by visiting professor and family for 3 months from July 1, 1970. Please contact Mr V. I. Paynter, Personnel Manager, Mullard Research Laboratories, Cross Oak Lane, Salfords, Redhill, Surrey (Telephone Horley 5544).

Wanted: From October 1, 1970, for 6 months, furnished 2 bedroom flat in London with easy public transportation access to the National Hospital, Queen Square, and to Hammersmith Hospital. Please contact Dr C. Bally, Biochemisches Institut der Universität, Zürichbergstr. 4, 8032 Zürich, Switzerland.

Vacant: Modern furnished apartment in Rome (historic centre, adjoining Palazzo Farnese) from September 1970 for 6 months or longer. 3 bedrooms, 2 bathrooms, terrace, central heating, automatic dishwasher. Please contact G. Wolf, Via Giulia 188, Rome 00186, Italy.

British Diary

Monday, June 22

Computing Techniques for Industrial Applications (four-day symposium) Hendon College of Technology, at The Burroughs, Hendon, London NW4.

Kinetic Methods in Analytical Chemistry (6.30 p.m.) Professor H. B. Mark (University of Michigan), Society for Analytical Chemistry, Electroanalytical Group, in the Mechanical Engineering Department, Imperial College, London SW7.

The Conformational Basis of Active Transport in the Mitochondrion (5 p.m.) Professor David E. Green (University of Wisconsin) University College London, in the Botany Theatre, Gower Street, London WC1.

Tuesday, June 23

Early Mammals (two-day symposium) Linnean Society of London, at Burlington House, Piccadilly, London W1.

Wednesday, June 24

Progress in the Application of Data Processing Techniques (2 p.m.) British Institute of Radiology, Nuclear Medicine Committee, in the Barnes Hall, Royal Society of Medicine, 1 Wimpole Street, London W1.

Protein Binding of Antibodies (2 p.m.) Dr G. Robinson, University of London, at the Royal Postgraduate Medical School, DuCane Road, London W12.

Thursday, June 25

Automation in Haematology (5.15 p.m.) Dr B. E. Gilliver, University of London, in the Page Street Lecture Theatre, Westminster Medical School, Horseferry Road, London SW1.

The Electrical Control of the Human Heart (5 p.m.) Dr Edgar Sowton, University of London, at the Institute of Cardiology, 35 Wimpole Street, London W1.

Trace Analysis (two-day meeting) Society for Analytical Chemistry, Scottish Section, jointly with the North East Section ; and the Atomic-Absorption Spectroscopy and the Radiochemical Methods Groups, at the University of St. Andrews, St. Andrews, Scotland.

Friday, June 26

The Earth, Professor Sydney Chapman, FRS; **The Sun and the Interplanetary Medium,** Professor D. E. Blackwell (11 a.m.–1 p.m.; **The Stars and the Structure of the Galaxy,** Sir Richard Woolley, OBE, FRS; **Extra-Galactic Nebulae,** Professor Sir Martin Ryle, FRS; **Astronomy of the Future,** Professor H. Bondi, FRS (2.15–6 p.m.), Royal Astronomical Society, at the Royal Institution, Albemarle Street, London W1 (Sesquicentenary Celebration Lectures).

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Universities Council for Adult Education. Report on the year 1968/1969. Pp. 12. (Oxford: F. W. Jessup, Honorary Secretary, University Council for Adult Education, The University, 1970.) 274

Bulletin of the British Museum (Natural History). Zoology. Vol. 19, No. 4: Fourteen New Species of Freeliving Marine Nematodes from the Exe Estuary. By Richard Martyn Warwick. Pp. 137–177. 24*s*. Vol. 19, No. 5: The Anatomy and Taxonomy of *Indostomus paradosus* Prashad and Mukerji. Pp. 179–209. 16*s*. (London: British Museum (Natural History), 1970.) [274

Cotton Research Corporation. Annual Report for 1969. Pp. 27. (London: Cotton Research Corporation, 1970.) [274

Ministry of Technology. Report by the Working Party on Maintenance Engineering. Pp. 14. (London: Ministry of Technology, 1970.) [274

Commonwealth Mycological Institute. Phytopathological Papers. No. 11: Banana Leaf Spot Disease (Sigatoka) Caused by *Mycosphaerella musicola* Leach. By D. S. Meredith. Pp. 147. 45*s*. No. 12: South American Leaf Blight (Microcyclus Ulei) of *Hevea brasiliensis*. By Paul Holliday. Pp. 31. 12*s*. (Kew: Commonwealth Mycological Institute, 1970.) [274

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 258, No. 821 (28 April, 1970): Further Observations on the Lower Devonian Plant *Gosslingia breconensis* Heard. By Dianne Edwards. Pp. 225–243. 18*s*; $2.35. Vol. 258, No. 822 (28 April, 1970): The Fine Structure of the Digestive Tubules of the Marine Bivalve *Cardium edule*. By G. Owen. Pp. 245–260. 27*s*; $3.50. (London: The Royal Society, 1970.) [284

Ministry of Technology and the Council of Engineering Institutions. Studies in Technological Manpower. No. 1: The Survey of Professional Engineers 1968. Pp. 57. 22*s* 6*d* (£1.12½) net. No. 2: The Survey of Professional Scientists 1968. Pp. 41. 15*s* (75*p*) net. (London: HMSO, 1970.) [284

Imperial Cancer Research Fund. Sixty-seventh Annual Report and Accounts, 1968/1969. Pp. 76. (London: Imperial Cancer Research Fund, 1970.) [284

The Zoological Record. 1967, Vol. 104, Section 1: Comprehensive Zoology. Compiled by S. Markowski. Pp. 31. 12*s* 6*d*. 1967, Vol. 104, Section 6: Vermes. Part 1 compiled by R. A. Bray, J. W. Coles and Stephen Prudhoe. Part 2 compiled by E. G. Easton. Part 3 compiled by S. Ware. Pp. 179. 60*s*. 1967, Vol. 104, Section 15: Pisces. Recent literature compiled by G. Palmer, Ann Proctor and David Cole. Fossil literature compiled by H. A. Toombs, R. Miles and C. Patterson. Pp. 163. 40*s*. 1968, Vol. 104, Section 17: Reptilia. Compiled by Lorna Goldsmith, Ann Proctor, David Cole and Michael Dadd. Pp. 108. 1967, Vol. 104, Section 18: Aves. Compiled by Carolyn Roberts and Dorothy Thelwall. Pp. 264. 60*s*. (London: Zoological Society of London, 1969 and 1970.) [284

Shirley Institute—Report and Accounts, 1st April to 30th September 1969. Pp. 22. (Didsbury, Manchester: The Cotton Silk and Man-Made Fibres Research Association, Shirley Institute, 1970.) [284

Education and Science in 1969: Being a Report of the Department of Education and Science. Pp. 164+36 photographs. (London: HMSO, 1970.) 20*s* net. [294

British Flame Research Committee. 1969 Annual Report. Pp. 20. (London: British Flame Research Committee, 1970.) [294

Report of the Rugby School Natural History Society for the year 1969. (One Hundred and Third Issue.) Pp. 26. (Rugby: Rugby School Natural History Society, 1970.) [304

Bulletin of the British Museum (Natural History). Mineralogy. Vol. 2, No. 5: Petrology of Lord Howe Island, Part 1: The Younger Volcanics. By P. M. Gale. Pp. 221–284 + plates 10–15. (London: British Museum (Natural History), 1970.) 45*s*. [304

Rubber and Plastics Research Association of Great Britain. Fiftieth Annual Report, 1969. Pp. 27. (Shawbury, Shrewsbury: Rubber and Plastics Research Association of Great Britain, 1970.) [304

British Medical Bulletin, Vol. 26, No. 2 (May 1970): Symposium on Recent Research on the Retina. Pp. 99–184. (London: The British Council, 1970.) 40*s*. [304

Civil Service Commission. Annual Report 1969. (103rd Report.) Pp. 65. (London: Civil Service Commission, 1970.) [304

University of Cambridge, Department of Engineering. Report of the Head of the Department for the year 1968/1969. Pp. 20. (Cambridge: The University, 1970.) [304

Progress in Nuclear Physics, Vol. 12, Pt. 1. Pp. 75. (Oxford, New York and Toronto: Pergamon Press, 1970.) 25*s*; $3.40. [304

Machinery Buyers' Guide 1970. 41st edition. Edited by Frederick A. J. Browne. Pp. 1440. (London: The Machinery Publishing Co., Ltd., 1970. Orders to Machinery Publishing Co., Ltd., New England House, New England Street, Brighton, BN1 4HN.) 25*s*. [304

The Reporter, Vol. 1, No. 1 (April 1970). Pp. 1–18. (London: Chelsea College, University of London, 1970.) [304

The NHS: Three Views. By Peter Draper, Maurice Kogan and J. N. Morris. (Fabian Research Series, No. 287.) Pp. 23. (London: Fabian Society, 1970.) 4*s* (20*p*). [15

Titles of Dissertations approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academical Year, 1968–1969. Pp. 39. (Cambridge: The University, 1970.) [15

University of Oxford. Report of the Committee on the University Press. (Supplement No. 7 to the *University Gazette*, Vol. C, May 1970.) Pp. 184. (Oxford: The University, 1970.) 20*s*. [15

Department of Education and Science; Scottish Education Department; Northern Ireland Ministry of Education; University Grants Committee. Education Statistics for the United Kingdom 1967. Pp. xxvii + 73. (London: HMSO, 1970.) 25*s* net. [15

Southern's Tool Catalogue: Tools and Equipment for Precision Industries. Pp. 30. (Orpington, Kent: Southern Watch and Clock Supplies, Ltd., 1970.) [15

The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1968. Pp. iii + 69. (Dublin: Medical Research Council of Ireland, 1970.) 5*s*. [15

Building Research Station. Current Paper 4/70: Load-Carrying Capacity of Slab-Strips Restrained Against Longitudinal Expansion. By E. H. Roberts. Pp. 12. (Garston, Watford: Building Research Station, 1970.) *Gratis*. [15

British Industrial Biological Research Association. Annual Report for 1969. Pp. 56. (Carshalton: British Industrial Biological Research Association, 1970.) [15

Other Countries

US Department of Commerce—National Bureau of Standards. Special Publication No. 320: Bibliography on Atomic Transition Probabilities, January 1916 through June 1969. By B. M. Miles and W. L. Wiese. Pp. viii + 104. (Washington, DC: Government Printing Office, 1970.) $1.25. [214

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APPOINTMENTS VACANT

UNIVERSITY OF EDINBURGH

DEPARTMENT OF ZOOLOGY

BIOCHEMIST

required as Research Associate in a three-year investigation of the role of proteins in plasma membrane structure, in the above Department. Previous experience in protein chemistry an advantage.

Salary scale: £1,355 to £1,605 per annum according to qualifications, together with F.S.S.U.

Applications, giving the names of two referees, should be sent to Dr. A. H. Maddy, Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT. Please quote reference 1060. (2426)

MEDICAL RESEARCH COUNCIL

VETERINARY MICROBIOLOGIST

(age 28-35) required for Scientific Staff of the Council's Laboratory Animals Centre. Candidates must be interested in laboratory animal medicine and be prepared to specialise in the microbiology and pathology of laboratory animals.

Previous experience in this new veterinary field would be an advanage in joining a team headed by a veterinary pathologist for whom the successful applicant will be expected to deputize when necessary.

Duties will include responsibility for the microbiological screening (including for some viruses) of germfree and specific pathogen free colonies, leading to the development of animals with a more accurately defined flora.

The post carries responsibility and offers unique opportunities for the successful applicant.

Excellent conditions of service. Starting salary normally within the range £1,175 to £2,275 and £2,455 to £2,930 in accordance with age, qualifications and experience; F.S.S.U., London allowance £60 ; six weeks annual leave.

Applications to the Director, M.R.C. Laboratory Animals Centre, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey.

(2311)

UNIVERSITY OF LONDON

CHAIR OF ZOOLOGY AT BEDFORD COLLEGE

The Senate invite applications for the Chair of Zoology tenable at Bedford College. Salary not less than £4,120 a year plus £100 London Allowance.

Applications (10 copies), must be received not later than July 10, 1970, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained. (2440)

CSIRO

POSTDOCTORAL RESEARCH FELLOW (CHEMICAL ENGINEER)

AUSTRALIA

DIVISION OF CHEMICAL ENGINEERING

GENERAL The Division of Chemical Engineering of the Commonwealth Scientific and Industrial Research Organization undertakes fundamental studies of selected unit operations and their application to the chemical and process industries, including general process development. The Division has recently taken occupation of extensive new laboratories at Clayton, Victoria, adjacent to Monash University, about 12 miles from Melbourne. Powerful analogue, digital and hybrid computing facilities are available.

DUTIES The appointee will be required to join teams engaged on work on one or other of the following fields:

(a) Studies of gas-solid fluidisation in large scale equipment. The work would involve experimental and theoretical studies inter alia of, the effect of distributor design on gas and solids motion in beds of up to 64 ft^3 cross section.

(b) Studies of heat transfer in relation to water desalination by distillation, food poisoning etc. Present work includes fundamental studies of boiling heat transfer in film flow, and heat transfer to oil/water systems.

QUALIFICATIONS Applicants should have a degree in chemical engineering or applied science, together with a Ph.D. degree or post-graduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability. Experience in one or other of the above fields would be of value, but is not essential.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The appointment will be initially for a fixed term of three years, after which, if mutually desired, either an extension of tenure or appointment for an indefinite period will be considered. The appointment will be conditional upon passing a medical examination and an initial probationary period of twelve months may be specified. An indefinite appointment carries with it Commonwealth Superannuation Fund or Provident Account privileges. Further details supplied on application.

Applications, (quoting Appointment No: 608/120), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64–78 Kingsway,
LONDON, WC2B 6BD. by the *7th August, 1970.*

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(2474)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

ASSISTANT LIBRARIAN

Applications are invited from graduates, with professional training and/or appropriate experience, or from non-graduates with A.L.A., for the post of Assistant Librarian (Science) in the College Library.

The salary will be either within the preliminary scale £1,200 to £1,355 per annum or £1,355 to £2,230 (bar) per annum according to qualifications and experience, with the prospect, in the case of graduates, of extension to £3,105 per annum.

Further particulars, quoting reference number B6 may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, Glam., SA2 8PP, by whom applications should be received not later than **Monday, July 13, 1970.**

(2503)

IMPERIAL COLLEGE

(UNIVERSITY OF LONDON)

DEPARTMENTAL LIBRARIAN

Applications, from graduates or others with relevant experience, are invited for the post of Librarian of the Biochemistry Department Library of Imperial College. The Library is shortly to move into a new building and will be run in conjunction with the Chemistry Department Library. The appointment will be made at the Assistant Librarian III Grade and the salary will be in the range £1,100 to £1,800 per annum, plus F.S.S.U.

Applications with full curriculum vitae and the names of two referees should be made as soon as possible to Professor Sir Ernst Chain, F.R.S., Department of Biochemistry, Imperial College, London S.W.7. (2502)

Overseas Development

The provision of skilled manpower is a vital element in Britain's aid to the developing countries. Your professional skills are needed overseas and you will have the satisfaction of doing a challenging, responsible and worthwhile job. Terms of service usually include substantial extra benefits including family passages, paid leave, educational grants and free or subsidised accommodation. For certain of these appointments an appointment grant of up to £200 and a car purchase loan of up to £300 may be payable. Salaries are assessed in accordance with qualifications and experience. In most cases the emoluments shown are based on local salary scales plus basic allowances. Appointments are on contract for 2-3 years in the first instance, unless otherwise stated. Candidates should be citizens of, and permanently resident in, the United Kingdom.

PRINCIPAL FISHERIES SCIENTIST
£2,645-4,375/Saudi Arabia

To participate in establishing and equipping a Marine Research Centre and to assist in planning and supervising a five year research programme as part of a team sponsored by the University College of North Wales and the Saudi Arabian Ministry of Agriculture and Water. Candidates must have a PhD in marine biology with at least three years' experience in fisheries research and preferably organisational experience in marine research and familiarity with tropical waters. Emoluments quoted above include a variable tax free overseas allowance of £645-1,375.

RESEARCH OFFICER (BIOLOGIST)
£1,754-2,669/East Africa

To conduct an investigation of the tunas of commercial importance in East African waters. He must have a good honours degree in a zoological subject and research experience in marine fisheries. Emoluments quoted above include an inducement allowance, normally tax free, of £732-926. A terminal gratuity of 25% of the total emoluments is also payable.

ASSISTANT CONSERVATOR OF FORESTS
£1,636-2,876/Malawi

To administer a forest area or undertake an assignment in any specialised branch of the Forest Department. He must have a degree in forestry. Emoluments quoted above include a supplement, normally tax free, of £216-276. A gratuity, normally tax free, of 25% of emoluments (excluding supplement) is payable on completion of tour of not less than 30 months.

FISHERIES BIOLOGIST
£3,160-4,410/Jamaica

To investigate aspects of fisheries biology, to recommend what fisheries legislation should be enacted and to propose an expansion plan for the fishing industry. He must have had formal training in Marine Biology with relevant experience in research activities. Emoluments quoted above include a variable tax free overseas allowance of £660-1,410.

FISHERIES OFFICER
£3,500-5,585/West Cameroon

To supervise jointly with Oxfam and the US Peace Corps the development of inland fish culture. He must have a degree in Natural Science with considerable experience in tropical inland fisheries. Emoluments quotes above include a variable tax free overseas allowance of £1,000-2,085.

FISHERIES TRAINING OFFICER
£2,686-3,354/Zambia

To lecture to students taking a Diploma in Fisheries. He must have a degree in Social Sciences with specialisation in fisheries biology or equivalent and relevant field experience in Africa. Emoluments quoted above include an inducement allowance, normally tax free, of £684-750. A gratuity, normally tax free, of 25% of total emoluments is also payable.

SENIOR FISHERIES OFFICER
£3,766/Zambia

To administer large staff and direct fisheries research programmes in the lakes and rivers of Zambia. He must have either a good honours degree in biology plus ten years experience, or a higher degree plus eight years experience. Experience in freshwater biological research necessary. Emoluments quoted above include an inducement allowance, normally tax free, of £780. A terminal gratuity, normally tax free, of 25% of total emoluments is also payable.

ASSISTANT CONSERVATORS OF FORESTS
£2,029-3,622/Sarawak

To undertake general forest administration. He must have a degree in Forestry; previous experience desirable. Emoluments quoted above include an inducement allowance of £804-1,303. A gratuity of approximately 20% of total emoluments is also payable.

MARKETING MANAGER
£3,766/Zambia

To organise and control the marketing of timber. He must have a degree in Commerce or Economics OR a Diploma of the Institute of Marketing or Management with at least 5 years relevant experience. Emoluments quoted above include an inducement allowance, normally tax free, of £780. A gratuity, normally tax free, of 25% of total emoluments is also payable.

ASSISTANT CONSERVATOR OF FORESTS
£1,666-2,556/Guyana

Required for general forestry duties. He must have a degree in forestry, preferably with previous experience. Emoluments quoted above include an inducement allowance, normally tax free, of £636-936. A gratuity of 25% of total emoluments is also payable.

BIOLOGISTS
£1,734-3,354/Zambia

To control anti-tsetse field operations in an assigned area, including administrative duties and scientific assessment of tsetse control measures. They must have an honours degree in a natural science or a relevant applied science in agriculture and forestry, preferably with previous experience of tsetse control. Emoluments quoted above include an inducement allowance, normally tax free, of £600-750. A gratuity, normally tax free of 25% of total emoluments is also payable.

ASSISTANT CONSERVATORS OF FORESTS
£1,734-3,354/Zambia

For general district duties; or forest surveys and the preparation of working plans; or silvicultural research. They must have a degree in forestry. Emoluments quoted above include an inducement allowance, normally tax free, of £600-750. A gratuity, normally tax free, of 25% of total emoluments is also payable.

MINISTRY OF OVERSEAS DEVELOPMENT

Further information about any of these vacancies may be obtained by writing briefly, stating your age, qualifications and experience, to:

The Appointments Officer, Room 301 N,
MINISTRY OF OVERSEAS DEVELOPMENT,
Eland House, Stag Place, London, S.W.1.

(2487)

Nutritionist

FOOD STANDARDS AND SCIENCE DIVISION

Ministry of Agriculture, Fisheries and Food, London

NUTRITIONIST (graded Senior Scientific Officer) to take responsibility for nutritional aspects of the National Food Survey. These include identifying and investigating related nutritional problems, and planning and conducting collaborative investigations of the effects of modern methods of production and processing on the nutritive value of foods.

Qualifications : 1st or 2nd class honours degree or equivalent qualification in Biochemistry, Physiology or Nutrition and at least three years' postgraduate experience.

Salary : £2,318–£2,828.

Age : Normally 26–31.

Non-contributory pension. Prospects of promotion.

Application forms are obtainable by writing to the Civil Service Commission, Savile Row, London, W1X 2AA, or by telephoning 01-734 6010, ext. 229 (after 5.30 p.m. 01-734 6464 " Ansafone " service).

Please quote S/52-53/70/MAF/43/G. Closing date 14 July, 1970.

(2472)

STATES OF GUERNSEY HORTICULTURAL ADVISORY SERVICE

PLANT PATHOLOGIST

Applications are invited for the above post. Applicants must have a University Degree in a biological science and postgraduate experience in plant pathology.

The successful applicant will take charge of a small plant pathology section concerned primarily with the diagnosis of disease and pest problems in glasshouse crops, advising growers on control measures and, to a limited extent, evaluating new chemicals in field trials.

The salary scale will be on a scale £1,660—£2,760 which is reviewed annually in August each year. Commencing salary will depend on age, qualifications and experience.

Consideration will be given to applicants' housing requirements. Removal expenses and fares are normally paid.

Application form and further details are available from the **Establishments Officer, Civil Service Board, Grange House, St. Peter Port, Guernsey.** Completed applications must be submitted not later than July 4, 1970.

(2489)

CITY OF YORK

APPOINTMENT OF KEEPER OF THE YORKSHIRE MUSEUM

(Salary : Senior Officers' Grade £1,870–£2,445)

Applications are invited for appointment as Keeper of the Yorkshire Museum, York, which houses valuable archaeological, geological and natural history collections.

Applicants should have a degree in archaeology, biology, geology or art history and preferably the Diploma of the Museums Association. Appropriate museum experience is essential.

Further information about the appointment from the Town Clerk, Guildhall, York, YO1 1QN, to whom applications should be sent by July 8, 1970. (2492)

UNIVERSITY OF WAIKATO

HAMILTON, NEW ZEALAND

SENIOR LECTURESHIPS AND LECTURESHIPS IN THE SCHOOL OF SCIENCE

The University of Waikato invites applications for Senior Lectureships and Lectureships in its new School of Science. Appointments to each position will be considered at either level, but not all appointments can be made at the Senior Lecturer level. The positions are as follows :

PHYSICAL CHEMIST, preferably with a teaching interest in thermodynamics and/or statistical mechanics.

PHYSICISTS with research interests in one of the following : the physics of the liquid state, geophysics, biophysics.

EARTH SCIENTIST to lecture in general geology including petrology, mineralogy and stratigraphy. Earth Sciences is planned to embrace studies dealing more particularly with the surface of the earth and at present includes courses in pedology, geology and geomorphology.

BIOLOGICAL SCIENTISTS with teaching and research interests in the following fields : plant physiology, animal physiology, genetics, population ecology.

Enquiries are invited in other fields about appointments which may be made in 1971 and 1972, with particular reference to the following fields : biochemistry, organic/biochemistry, microbiology, soil chemistry, geophysics and biophysics.

The University of Waikato started teaching Science in 1970 and has 130 first year science students and 14 D.Phil. students. Second and fourth year courses will be introduced in 1971 and third year courses in 1972.

The policy of the School of Science is to encourage inter-disciplinary research projects and research on problems of local interest.

As well as the School of Science, the University has Schools of Humanities, Social Sciences, and Education. Some science courses are available to students in these Schools. The University occupies a pleasant site of about 130 acres in the city of Hamilton.

Salary scales are under review. At present, salaries are as follows :

Senior Lecturers : $NZ4,700-$NZ5,500-$NZ6,600.

Lecturers : $NZ3,100-$NZ4,800.

Conditions of appointment are available from the Registrar of any university in New Zealand and from the Secretary of the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, WC1H OPF.

Applications close on July 20, 1970.

(2480)

UNIVERSITY OF DURHAM

DEPARTMENT OF PSYCHOLOGY

Applications are invited for the post of COMPUTER SUPERVISOR in the Department of Psychology from October 1, 1970, or such later date as may be arranged. The person appointed will be responsible for the management of the Department's IBM 1130 on-line computing system and the preparation of programmes.

Candidates should be of graduate or equivalent standing and have experience of computer room practice and programming. Salary will be on the scale: £1,355 by £125 to £2,605 per annum with F.S.S.U., according to experience and qualifications.

Applications (3 copies) together with the names and addresses of three referees, should be sent by Monday, July 6, 1970, to the Registrar and Secretary, Old Shire Hall, Durham, from whom further particulars may be obtained. (2452)